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(57) Abstract

Novel fusion polypeptide ligands that bind Eph family receptors or the Tie-2 receptor are identified, and methods for making the fusion polypeptide ligands in biologically active form are described. Nucleic acids encoding these novel fusion polypeptide ligands enable production of the fusion polypeptide ligands. The method of making the nucleic acids and the fusion polypeptide ligands is broadly applicable to the production of polypeptide ligands in general, resulting in improved affinity and/or increased activity of the ligand when compared to its native form.

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METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

This application claims priority of U.S. Application No. 60/113,387, filed December 23, 1998. Throughout this application, various publications are cited. The disclosures of each and all of those publications are hereby incorporated by reference in their entireties into this application.

INTRODUCTION

The present invention provides for novel methods for producing novel fusion polypeptide ligands that have enhanced biological activity as compared to the polypeptide ligands in their native form. The invention also provides for nucleic acids useful for producing biologically active fusion polypeptide ligands, and the fusion polypeptide ligands themselves.

BACKGROUND OF THE INVENTION

The ability of polypeptide ligands to bind cells and thereby elicit a phenotypic response such as cell growth, survival or differentiation is often mediated through transmembrane tyrosine kinases. The extracellular portion of each receptor tyrosine kinase (RTK) is generally the most distinctive portion of the molecule, as it provides the protein with its ligand-recognizing characteristic. Binding of a ligand to the extracellular domain results in signal transduction via an intracellular tyrosine kinase catalytic domain which transmits a biological signal to intracellular target proteins. The particular array of sequence motifs of this cytoplasmic, catalytic domain determines its access to potential kinase substrates (Mohammadi, et al.,1990, Mol. Cell. Biol., 11: 5068-5078; Fantl, et al., 1992, Cell, 69:413-413).

RTKs appear to undergo dimerization or some related conformational change following ligand binding (Schlessinger, J., 1988, Trend Biochem. Sci.

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13:443-447; Ullrich and Schlessinger, 1990, Cell, 61:203-212; Schlessinger and Ullrich, 1992, Neuron 9:383-391); molecular interactions between dimerizing cytoplasmic domains lead to activation of kinase function. In some instances, such as the growth factor platelet derived growth factor (PDGF), the ligand is a dimer that binds two receptor molecules (Hart, et al., 1988, Science, 240: 1529-1531; Heldin, 1989, J. Biol. Chem. 264:8905-8912) while, for example, in the case of EGF, the ligand is a monomer (Weber, et al., 1984, J. Biol. Chem., 259:14631-14636).

Throughout the history of the biotechnology industry, many novel genes and associated proteins have been identified by virtue of their sequence homology with known genes. Many such proteins are purported to be receptors, but since their cognate ligands have not been identified, they are referred to as orphan receptors. The screening of many of these orphan receptors often leads to the identification of ligands that are capable of binding to the receptor, although the binding is often not associated with activation of any intracellular kinases or any other phenotypic change. Such was the case for members of the Eph receptor family. For sake of clarity, applicants incorporate by reference herein a letter cited as Eph Nomenclature Committee, 1997, published in Cell vol. 90: 403-403 (1997) which sets forth a nomenclature for the Eph Receptor and Eph Ligand Families.

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Little, if any, biological activity had been observed in response to binding of a ligand to an Eph family receptor prior to the discovery as set forth in U.S. Patent No. 5,747,033 issued May 5, 1998. That patent describes the concept of "clustering" whereby the soluble domains of ligands were combined to create multimers capable of activating the cognate receptors. Applicants have now extended the concept of clustering to additional ligands outside the Eph family, for example, the Tie-2 receptor ligands known as the angiopoietins, and have also discovered that this method for production of homogeneous forms of clustered ligands is broadly applicable to improve

the affinity and/or increase the activity of a ligand as compared to the native form of the ligand.

Angiopoietin-1 (Ang) is one of two known ligands for the Tie-2 receptor and has been shown to be an agonist for Tie-2 (Davis, et al, 1996, Cell 87:1161-1169), whereas the second known ligand, angiopoietin-2, has been shown to be a naturally occurring antagonist of the Tie-2 receptor (Maisonpierre, et al., 1997, Science 277:55-60). Ang1* is a mutant form of angiopoietin-1 that comprises the N-terminal domain of angiopoietin-2 fused to the coiled-coil domain and the fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. Ang1* has been shown to be a potent agonist for the Tie-2 receptor.

Experiments with mutants of angiopoietin-1 and angiopoietin-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions of, for example Ang-1-FD-Fc, (i.e., the fibrinogen domain of Ang-1 fused to an Fc domain), can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD (dimerization occurs due to the interaction between the Fc components of adjacent molecules). However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD (i.e., the fibrinogen domain of Ang-2) were designed that were intrinsically more highly clustered.

SUMMARY OF THE INVENTION

The present invention provides for novel, biologically active, soluble forms
of polypeptide ligands that bind to receptors on cells. Such polypeptide
ligands are useful in promoting a differential function and/or influencing
the phenotype, such as growth and/or proliferation, of receptor-bearing

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cells. The invention also provides for nucleic acids encoding such polypeptide ligands, and both prokaryotic and eukaryotic expression systems for producing such polypeptide ligands. According to the invention, soluble forms of the polypeptide ligands described herein may be used to promote biological responses in receptor-expressing cells. In particular, a general method is described herein which produces fusion polypeptide ligands that may then be clustered, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

DESCRIPTION OF THE FIGURES

<u>Figure 1A-1E -</u> Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-Fc.

Figure 2A-2E - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-FD-Fc.

<u>Figure 3A-3E</u> - Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-Fc-FD.

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<u>Figure 4A-4E</u> - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-Fc-FD.

Figure 5 - Molecular Weight Analysis of Ang-1-FD-Fc-FD protein. SDS

PAGE analyses showing a band running at about 210kD under non-reducing conditions (lane 3) and a band running at about 85kD under reducing conditions (lane 7).

Figure 6 - Light scatter analysis to confirm the molecular weight of Ang-1-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is homogenous.

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Figure 7 - Molecular Weight Analysis of Ang-2-FD-Fc-FD. SDS PAGE analyses showing a band running at about 200kD under non-reducing conditions (lanes 7 and 8) and a band running at about 88kD under reducing conditions (lanes 3 and 4).

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Figure 8 - Light scatter analysis to confirm the molecular weight of Ang-2-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 171kD and presence of a single peak implies that the

protein solution is homogenous.

<u>Figure 9</u> - Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926.

Figure 10 - Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

Figure 11 - Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, at a 20 fold molar excess, angiopoietin-2 is not able to reduce the Ang1*-mediated phosphorylation level to 50%. This result, coupled with the results described in Figure 10 implies that Ang-2-FD-Fc-FD is a more potent inhibitor of Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.

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Figure 12 - Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10.

Figure 13 - Ability of angiopoietin-2 to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at

blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11.

<u>Figure 14A-14E</u> - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B1-Ephrin-B1-Fc.

<u>Figure 15A-15E</u> - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B2-Ephrin-B2-Fc.

Figure 16 - Comparison of Ephrin-B1-Fc, Ephrin-B1-Ephrin-B1-Fc, Ephrin-B2-Fc and Ephrin-B2-Ephrin-B2-Fc in standard EphB2 phosphorylation assays. COS cells were serum-starved and then left untreated (UT), lane 1, or were treated with unclustered and clustered Ephrin-B1-Fc (Efn-B1), lanes 2 and 3. COS cells were also treated with unclustered and clustered Ephrin-B1-Ephrin-B1-Fc (Efn-B1 DD), lanes 4 and 5. In addition cells were likewise treated with unclustered and clustered Ephrin-B2-Fc (Efn-B2), lanes 6 and 7 and with unclustered and clustered Ephrin-B2-Ephrin-B2-Fc (Efn-B2 DD), lanes 8 and 9. The extent of EphB2 phosphorylation was assessed by anti-phosphotyrosine western blotting (upper panels) and the relative amounts of EphB2 in each lane was determined by anti-EphB2 western blotting (lower panels).

Figure 17 - Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were stimulated with 0.4 μg/ml Ang1* or 0.2 μg/ml or 0.4 μg/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells.

Figure 18 - Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were treated with 0.2

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 μ g/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 μ g/ml, 4 μ g/ml, 8 μ g/ml or 16 μ g/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. Ang-2-FD-Fc-FD is able to block or stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

DETAILED DESCRIPTION OF THE INVENTION

As described in greater detail below, applicants have discovered a method for "clustering" polypeptide ligands, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

The present invention provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

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In one embodiment of the invention, the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand. The first and second subunits may each have one or more than one copy of the receptor binding domain of the ligand. In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. Alternatively, the

receptor binding domain is from a ligand selected from the group consisting of the EPH family of ligands (i.e., the ephrins).

In another embodiment of the invention, the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit. For example, the first subunit may comprise the receptor binding domain of an angiopoietin and the second subunit may comprise the receptor binding domain of vascular endothelial growth factor (VEGF). Alternatively, the first subunit may comprise the receptor binding domain of VEGF and the second subunit may comprise the receptor binding domain an angiopoietin. Still further, the first and second subunits may each have one or more than one copy of the receptor binding domain of their respective ligand.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as $Fc(\Delta C1)$.

The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996

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and include <u>S. cerevisiae</u> repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) <u>103</u>:321-326); the <u>S. cerevisiae</u> type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. <u>5</u>:3381-3390); the <u>S. calsbergensis</u> alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene <u>36</u>:333-340); and the <u>Neurospora crassa</u> ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. <u>262</u>:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell <u>29</u>:671-679); the <u>S. cerevisiae SUC2</u> gene (Carlson et al., 1983, Mol. Cell. Biol. <u>3</u>:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

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Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as <u>E. coli</u>, a yeast cell, such as <u>Pichia pastoris</u>, an insect cell, such as <u>Spodoptera frugiperda</u>, or a mammalian cell, such as a COS or CHO cell.

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The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

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The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system.

The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The present invention also provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component. In one embodiment of the invention, the receptor binding domains are fused contiguously. In another embodiment of the invention, the receptor binding domains are from a ligand that is not a member of the EPH family of ligands (i.e., not an ephrin). In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. In an alternative embodiment, the receptor binding domain is from vascular endothelial growth factor (VEGF). In another embodiment, the receptor binding domain is from an ephrin.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

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In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as $Fc(\Delta C1)$.

The present invention also provides for fusion polypeptides encoded by the 10 nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996 and include S. cerevisiae repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) 103:321-326); the S. cerevisiae type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. 5:3381-3390); the S. calsbergensis alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene 36:333-340); and the Neurospora crassa ornithine decarboxylase (Digangi, et al., 1987, J. Biol. 20 Chem. 262:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); the S. cerevisiae SUC2 gene (Carlson et al., 1983, Mol. Cell. Biol. 3:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc 25 domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is

operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as <u>E. coli</u>, a yeast cell, such as <u>Pichia pastoris</u>, an insect cell, such as <u>Spodoptera frugiperda</u>, or a mammalian cell, such as a COS or CHO cell.

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system. The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

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The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The Examples describe the preparation of novel polypeptide ligands that comprise a receptor binding domain of a member of the Eph (Eph transmembrane tyrosine kinase family ligands) family of ligands or of a

member of the angiopoietin family of ligands that can bind the Tie-2 receptor.

For a description of novel Eph family ligands, methods of making and using them, as well as the sequences of EHK-1L, B61 and ELK-L, together with a description of a method of enhancing the biological activity of EPH family ligands by clustering them, applicants refer to U.S. Patent No. 5,747,033 issued on May 5, 1998 which is hereby incorporated by reference in its entirety. Applicants further refer to International Application PCT/US93/10879, published as WO 94/11020 on May 26, 1994; and International Application PCT/US96/17201 published as WO 97/15667 entitled "Biologically Active EPH Family Ligands" each of which is hereby incorporated by reference in its entirety.

As has been previously reported, a family of ligands for the TIE-2 receptor has been discovered and named the Angiopoietins. This family, consisting of TIE-2 ligand 1 (Ang-1); TIE-2 ligand 2 (Ang-2); TIE ligand 3 (Ang-3); and TIE ligand 4 (Ang-4) has been extensively characterized. For a description of the cloning, sequencing and characterization of the angiopoietins, as well as for methods of making and uses thereof, including the production and characterization of modified and chimeric ligands thereof, reference is hereby made to the following publications, each of which is incorporated by reference herein in its entirety: U.S. Patent No. 5,521,073 issued May 28, 1996; U.S. Patent No. 5,643,755 issued July 1, 1997; U.S. Patent No. 5,650,490 issued July 22, 1997; U.S. Patent No. 5,814,464 issued September 29, 1998; U.S. 25 Patent No. 5,879,672 issued March 9, 1999; U.S. Patent No. 5,851,797 issued December 22, 1998; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof," published as WO 96/11269 on 18 April 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled "TIE-2 Ligands Methods of Making and 30 Uses Thereof,"published as WO 96/31598 on 10 October 1996 in the name of

Regeneron Pharmaceuticals, Inc.; PCT International Application entitled

"TIE-2 Receptor Ligands (TIE Ligand-3; TIE Ligand-4) And Their Uses," published as WO 97/48804 on 24 December 1997 in the name of Regeneron Pharmaceuticals, Inc; and PCT International Application entitled "Modified TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc.

When used herein, fusion polypeptide includes functionally equivalent molecules in which amino acid residues are substituted for residues within the sequence resulting in a silent or conservative change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent or conservative alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc.

Cells that express the fusion polypeptides of the invention are genetically engineered to produce them by, for example, transfection, transduction, electroporation, or microinjection.

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The present invention encompasses the nucleic acid sequences encoding the fusion polypeptides of the invention, as well as sequences that hybridize under stringent conditions to nucleic acid sequences that are complementary to the nucleic acid sequences of the invention. Stringent conditions are set forth in, for example, Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). In addition, the present invention encompasses nucleic acid sequences that are different from the nucleic acid sequences of the invention but which nevertheless encode the fusion polypeptides of the invention due to the degeneracy of the genetic code.

In addition, the present invention contemplates use of the fusion polypeptides described herein in tagged forms.

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Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion polypeptides of the invention using appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of nucleic acid sequence encoding the fusion polypeptides of the invention may be regulated by a second nucleic acid sequence so that the fusion polypeptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the fusion polypeptides described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the fusion polypeptide include, but are not limited to the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980,

Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the b-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. <u>75</u>:3727-3731), or the <u>tac</u> promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) 10 promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell <u>38</u>:647-658; Adames et al., 1985, Nature <u>318</u>:533-538; Alexander et al., 1987, Mol. Cell. Biol. <u>7</u>:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et 20 al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. <u>5</u>:1639-1648; Hammer et al., 1987, Science <u>235</u>:53-58); alpha 1-antitrypsin 25 gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature <u>315</u>:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); 30 myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene

control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising Eph fusion polypeptide encoding or angiopoietin fusion polypeptide encoding nucleic acids as described herein, are used to transfect the host and thereby direct expression of such nucleic acid to produce fusion polypeptides which may then be recovered in biologically active form. As used herein, a biologically active form includes a form capable of binding to the relevant receptor and causing a differentiated function and/or influencing the phenotype of the cell expressing the receptor. Such biologically active forms would, for example, induce phosphorylation of the tyrosine kinase domain of the Ehk-1, Elk, or Tie2 receptor, or stimulation of synthesis of cellular DNA.

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Expression vectors containing the nucleic acid inserts can be identified by three general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign nucleic acids inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted nucleic acid sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign nucleic acid sequences in the vector. For example, if an efl nucleic acid sequence is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign nucleic acid product expressed by the recombinant. Such assays can be based, for example, on the physical or

functional properties of the nucleic acid product of interest, for example, by binding of a ligand to a receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or binding to antibodies produced against the protein of interest or a portion thereof.

Cells of the present invention may transiently or, preferably, constitutively and permanently express the ephrin or angiopoietin fusion polypeptide as described herein.

The ephrin fusion polypeptides of the invention may be useful in methods of treating a patient suffering from a neurological disorder comprising treating the patient with an effective amount of the ephrin fusion polypeptide.

For example, the Elk receptor is expressed primarily in brain. Accordingly, it is believed that an Elk binding ephrin fusion polypeptide ligand will support the induction of a differential function and/or influence the phenotype, such as growth and/or survival of neural cells that express this receptor.

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The present invention also provides for pharmaceutical compositions comprising the ephrin fusion polypeptide in a suitable pharmacologic carrier. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

As our understanding of neurodegenerative disease/neurotrauma becomes clearer, it may become apparent that it would be beneficial to decrease the effect of endogenous Efl-6. Therefore, in areas of nervous system trauma, it may be desirable to provide Efl-6 antagonists, including, but not limited to,

fusion polypeptide forms of Efl-6 which may compete with cell-bound ligand for interaction with Elk receptor. It may be desirable to provide such antagonists locally at the injury site rather than systemically. Use of an Efl-6 antagonist providing implant may be desirable.

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Alternatively, certain conditions may benefit from an increase in Efl-6 responsiveness. It may therefore be beneficial to increase the number or binding affinity of Efl-6 in patients suffering from such conditions.

The invention herein further provides for the development of a fusion polypeptide, as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE-2 receptor. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

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Because TIE-2 receptor has been identified in association with endothelial cells and, as was previously demonstrated, blocking of agonists of the receptor such as TIE-2 ligand 1 (Ang-1) has been shown to prevent vascularization, applicants expect that TIE-2 agonist fusion polypeptides of the invention may be useful for the induction of vascularization in diseases or disorders where such vascularization is indicated. Such diseases or disorders would include wound healing, ischemia and diabetes. The ligands may be tested in animal models and used therapeutically as described for other agents, such as vascular endothelial growth factor (VEGF), another endothelial cell-specific factor that is angiogenic. Ferrara, et al. U.S. Patent No. 5,332,671 issued July 26, 1994. The Ferrara reference, as well as other studies, describe in vitro and in vivo studies that may be used to demonstrate the effect of an angiogenic factor in enhancing blood flow to ischemic myocardium, enhancing wound healing, and in other therapeutic settings wherein neoangiogenesis is desired. [see Sudo, et al., European Patent Application 0 550 296 A2 published July 7, 1993; Banai, et al. Circulation 89:2183-2189 (1994); Unger, et al. Am. J. Physiol. 266:H1588-H1595

(1994); Lazarous, et al. Circulation 91:145-153 (1995)]. According to the invention, the agonist fusion polypeptides may be used alone or in combination with one or more additional pharmaceutically active compounds such as, for example, VEGF or basic fibroblast growth factor (bFGF).

Conversely, antagonists of the TIE-2 receptor, such as TIE-2 receptorbodies or TIE-2 ligand 2 (Ang-2) as described in Example 9 in International Publication No. WO 96/31598 published 10 October 1996, have been shown to prevent or attenuate vascularization, and are thus expected to be useful in preventing or attenuating, for example, tumor growth. Similarly then, TIE-2 antagonist fusion polypeptides of the invention would also be useful for those purposes. These antagonists may be used alone or in combination with other compositions, such as anti-VEGF antibodies, that have been shown to be useful in treating conditions in which the therapeutic intent is to block angiogenesis.

For example, applicants have determined that TIE-2 ligands are expressed in cells within, or closely associated with, tumors. For example, TIE-2 ligand 2 (Ang-2) appears to be tightly associated with tumor endothelial cells. Accordingly, TIE-2 antagonist fusion polypeptides of the invention may also be useful in preventing or attenuating, for example, tumor growth.

In other embodiments, the TIE-2 agonist fusion polypeptides of the invention described herein may be used as hematopoietic factors. A variety of hematopoietic factors and their receptors are involved in the proliferation and/or differentiation and/or migration of the various cells types contained within blood. Because the TIE-2 receptors are expressed in early hematopoietic cells, the TIE-2 ligands are expected to play a comparable role in the proliferation or differentiation or migration of these cells. Thus, for example, TIE-2 agonist fusion polypeptide compositions may be prepared, assayed, examined in <u>in vitro</u> and <u>in vivo</u> biological systems and

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used therapeutically as described in any of the following: Sousa, U.S. Patent No. 4,810,643, Lee, et al., Proc. Natl. Acad. Sci. USA 82:4360-4364 (1985) Wong, et al. Science, 228:810-814 (1985); Yokota, et al. Proc. Natl. Acad. Sci (USA) 81:1070 (1984); Bosselman, et al. WO 9105795 published May 2, 1991 entitled "Stem Cell Factor" and Kirkness, et al. WO 95/19985 published July 27, 1995 entitled "Haemopoietic Maturation Factor". Accordingly, the fusion polypeptides may be used to diagnose or treat conditions in which normal hematopoiesis is suppressed, including, but not limited to anemia, thrombocytopenia, leukopenia and granulocytopenia. In a preferred embodiment, the fusion polypeptides may be used to stimulate differentiation of blood cell precursors in situations where a patient has a disease, such as acquired immune deficiency syndrome (AIDS) which has caused a reduction in normal blood cell levels, or in clinical settings in which enhancement of hematopoietic populations is desired, such as in conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment or chemotherapy.

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The fusion polypeptides of the present invention may be used alone, or in combination with another pharmaceutically active agents such as, for example, cytokines, neurotrophins, interleukins, etc. In a preferred embodiment, the fusion polypeptides may be used in conjunction with any of a number of factors which are known to induce stem cell or other hematopoietic precursor proliferation, or factors acting on later cells in the hematopoietic pathway, including, but not limited to, hemopoietic maturation factor, thrombopoietin, stem cell factor, erythropoietin, G-CSF, GM-CSF, etc.

In an alternative embodiment, TIE-2 receptor antagonist fusion polypeptides are used to diagnose or treat patients in which the desired result is inhibition of a hematopoietic pathway, such as for the treatment of myeloproliferative or other proliferative disorders of blood forming organs

such as thrombocythemias, polycythemias and leukemias. In such embodiments, treatment may comprise use of a therapeutically effective amount of the fusion polypeptides as described herein.

Effective doses useful for treating these or other diseases or disorders may be 5 determined using methods known to one skilled in the art [see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975)]. Pharmaceutical compositions for use according to the invention include the fusion polypeptides described above in a pharmacologically acceptable 10 liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation prior to administration in vivo. For example, the pharmaceutical composition may comprise a fusion polypeptide in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, 20 subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

Administration may result in the distribution of the active agent of the
invention throughout the body or in a localized area. For example, in some
conditions which involve distant regions of the nervous system,
intravenous or intrathecal administration of agent may be desirable. In
some situations, an implant containing active agent may be placed in or
near the lesioned area. Suitable implants include, but are not limited to,
gelfoam, wax, or microparticle-based implants.

The present invention also provides for pharmaceutical compositions comprising the fusion polypeptides described herein, in a pharmacologically acceptable vehicle. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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EXAMPLES

Angiopoietin ligands:

As described *supra*, experiments with mutants of Ang-1 and Ang-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions (dimerization occurs due to the interaction between the Fc components of adjacent molecules), for example Ang-1-FD-Fc, can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD. However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD were designed that were intrinsically more highly clustered.

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Two general types of nucleic acid molecules were constructed. The first type consisted of two tandem copies of Ang-1-FD fused to an Fc tag, thus leading

to a secreted polypeptide molecule that is dimeric with respect to the Fc tag but tetrameric with respect to Ang-1-FD. Similarly, two tandem copies of Ang-2-FD fused to an Fc tag constituted the angiopoietin-2 version of this type of construct. These molecules were designated Ang-1-FD-FD-Fc and Ang-2-FD-FD-Fc, respectively.

In the second type of nucleic acid molecule constructed, two copies of Ang-1-FD were connected by an Fc tag bridging between them, thus creating the structure Ang-1-FD-Fc-FD that is still dimeric with respect to the Fc, as well as tetrameric with respect to Ang-1-FD. An angiopoietin-2 version was also constructed and these two molecules were designated Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD, respectively.

For either type of construct, similar properties were observed: unlike dimeric Ang-1-FD-Fc, which fails to activate Tie-2 in endothelial cells, both Ang-1-FD-Fc and Ang-1-FD-Fc-FD could readily activate Tie-2 in endothelial cells, with a potency comparable to that of the native ligand. Also, like native angiopoietin-2, Ang-2-FD-Fc-FD could antagonize angiopoietin-1 activity with a potency that is comparable to that of native angiopoietin-2, and with much greater potency than the marginally antagonistic activity of the Ang-2-FD-Fc dimer.

Construction of mutant angiopoietin nucleic acid molecules.

All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into the mammalian expression vector pMT21 (Genetics

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Institute, Inc.) with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5' end to promote protein translation. The bridging sequences described *infra* were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

Example 1: Construction of the Ang-1-FD-Fc, Ang-2-FD-Fc, Ang-1-FD-Fc-FD, and Ang-2-FD-Fc-FD nucleic acid molecules.

Ang-1-FD-Fc: Ang-1-FD-Fc consists of a trypsin signal sequence at its amino terminus to allow for secretion (bases 1-45 of Figure 1A) followed by the angiopoietin-1 fibrinogen domain (FD) (bases 46-690 of Figure 1A-Figure 1B), a short bridging sequence consisting of the amino acids Gly-Pro Ala-Pro (bases 691-702 of Figure 1B), a second angiopoietin-1 FD (bases 703-1750 of Figure 1B-Figure 1D), another bridging sequence consisting of the amino acids Gly-Pro-Gly (bases 1351-1359 of Figure 1D), and the coding sequence for the Fc portion of human IgG1 (bases 1360-2058 of Figure 1D-Figure 1E).

Ang-2-FD-FC: The Ang-2-FD-FC nucleic acid molecule was similarly constructed. It consists of a trypsin signal sequence (bases 1-45 of Figure 2A), an angiopoietin-2 FD (bases 46-690 of Figure 2A- Figure 2B), a bridging amino acid sequence Gly-Gly-Pro-Ala-Pro (bases 691-705 of Figure 2B), a second angiopoietin-2 FD (bases 706-1353 of Figure 2B-Figure 2D), another bridging amino acid sequence Gly-Pro-Gly (bases 1354-1362 of Figure 2D), and the coding sequence for the Fc portion of human IgG1 (bases 1363-2061 of Figure 2D-Figure 2E.

Ang-1-FD-Fc-FD: The Ang-1-FD-Fc-FD consists of a trypsin signal sequence (bases 1-45 of Figure 3A), an angiopoietin-1 FD (bases 46-690 of Figure 3A-3B), the bridging amino acid sequence Gly-Pro-Gly (bases 691-699 of Figure

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3B), the coding sequence for the Fc portion of human IgG1 (bases 700-1395 of Figure 3B-3D), another bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1396-1419 of Figure 3D), and a second angiopoietin-1 FD (bases 1420-2067 of Figure 3D-Figure 3E).

Ang-2-FD-Fc-FD: The Ang-2-FD-Fc-FD nucleic acid molecule consists of a trypsin signal sequence (bases 1-45 of Figure 4A), an angiopoietin-2 FD domain (bases 46-690 of Figure 4A-Figure 4B), the bridging amino acid sequence Gly-Gly-Pro-Gly (bases 691-702 of Figure 4B), the coding sequence for the Fc portion of human IgG1 (bases 703-1398 of Figure 4B- Figure 4D), the bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1399-1422 of Figure 4D), and a second angiopoietin-2 FD (bases 1423-2067 of Figure 4D-Figure 4E).

Example 2: Characterization of Ang-1 FD-Fc-FD protein.

Molecular Weight Analysis: The predicted molecular weight for Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses of COS cell-derived protein described infra confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions (Figure 5). Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To

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determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. Figure 6 shows the results of this analysis using COS cell-derived protein. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is, in fact, homogenous.

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang1-FD-Fc-FD DNA construct described supra. All transfections were performed using standard techniques known in the art. The COS cell supernatant was analyzed using Biacore technology (Pharmacia, Inc.) to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 765, which is equivalent to 0.9mg of recombinant protein/liter of COS cell supernatant. These values represent very high levels of expression.

<u>Purification of COS Supernatants</u>: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). In fact, the relative ease of purification of the Ang-1-FD-Fc-FD protein gives it a distinct advantage over the parent protein, angiopoietin-1, from which

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it is derived, and the mutant version of angiopoietin-1 called Ang1* that consists of the N-terminal of angiopoietin-2 fused to the coiled-coil domain and fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. (See PCT International Application entitled "Modified TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc., especially Figure 27, which is hereby incorporated by reference).

Both angiopoietin-1 and Ang1* require extensive, expensive and laborintensive purification schemes that result in relatively poor yields of recombinant protein. The need for cost-effective, simple purification schemes for biologicals intended for clinical use can not be overemphasized.

The COS cell supernatant was purified as described *supra* and yielded approximately 1 mg of purified Ang-1-FD-Fc-FD protein that was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of COS cell-derived Ang-1-FD-Fc-FD protein:

Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. This was of concern because the mutant molecule, Ang1*, has a history of containing between 10-20% N-terminally truncated species. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

Receptor binding analysis of COS cell-derived Ang-1-FD-Fc-FD: Previous
studies have determined that the fibrinogen domain (FD) of the
angiopoietin molecules is necessary for ligand/receptor interaction.
Furthermore, in order for high affinity binding to the Tie-2 receptor to

occur, native angiopoietin-1, angiopoietin-2, and the mutant Ang1* must form at least tetrameric, and possibly higher order, multimers. To determine whether the COS cell-derived Ang-1-FD-Fc-FD protein, which is tetrameric with respect to the FD domain, could bind to Tie-2 with high affinity, standard Biacore analysis was performed. Briefly, Tie-2-Fc receptor body protein, which is a fusion protein comprising the ectodomain of Tie-2 fused to the Fc domain of human IgG1, was immobilized on a Biacore chip. Ang-1-FD-Fc-FD-containing solution was passed over the chip and binding between Tie-2 ectodomain and Ang-1-FD-Fc-FD was allowed to occur. The binding step was followed by a 0.5 M NaCl high salt wash. The high salt wash was not able to disrupt the interaction between the Ang-1-FD-Fc-FD protein and the Tie-2 receptor ectodomain, implying that there is a strong interaction between the mutant ligand and receptor. This result is consistent with earlier Biacore results in which both Ang-1-FD-Fc-FD parent molecule, angiopoietin-1 and the mutant Ang1* molecule, have been shown to interact strongly with the Tie-2-Fc receptor and that this interaction is not disrupted by high salt. In contrast, several mutant molecules derived from the parent angiopoietin-1 molecule are readily dissociated from the Tie-2-Fc receptor when treated with high salt. The mutant molecules, designated Ang-1/FD (a monomer with respect to the FD), Ang-1/FD-Fc (also a monomer with respect to the FD, but which is able to form a dimer due to the presence of the Fc domain), and Ang-1/C/FD (a monomer with respect to the FD, but which also contains the coiled-coil domain of angiopoietin-1), do not exist in multimeric forms sufficient for high affinity binding to the Tie-2 receptor.

Example 3: Characterization of COS cell-derived Ang-2-FD-Fc-FD protein.

Molecular Weight Analysis: As described for Ang-1-FD-Fc-FD supra, the predicted molecular weight for Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form of Ang-2-FD-Fc-FD has a predicted

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weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104.

SDS PAGE analyses of COS cell-derived protein confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 88kD under reducing conditions (Figure 7). Light scatter analysis confirmed the molecular weight (171kD) and revealed that the Ang-2-FD-Fc-FD protein, like Ang-1-FD-Fc-FD, exists as a homogeneous species (Figure 8).

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang-2-FD-Fc-FD DNA construct described supra. The COS cell supernatant was analyzed by Biacore to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 606, which is equivalent to 0.7mg of recombinant protein/liter of COS cell supernatant. These values represent relatively high levels of expression.

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Purification of COS Supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The COS cell supernatant was purified as described for Ang-1-FD-Fc-FD supra and yielded approximately 2 mg of purified Ang-2-FD-Fc-FD protein that was used in the studies described *infra* to further characterize this protein.

N-terminal sequencing: Purified COS cell-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Glu, wherein X is Cys. This

sequence can be found at amino acids 16-20 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Receptor binding analysis of COS cell-derived protein: To determine whether the COS cell-derived Ang-2-FD-Fc-FD protein could bind to the Tie-2 receptor, standard Biacore analysis was performed as described for Ang-1-FD-Fc-FD supra. As with Ang-1-FD-Fc-FD, a high salt wash was not able to disrupt the interaction between the Ang-2-FD-Fc-FD protein and the Tie-2-Fc receptor, again implying that there is a strong interaction between mutant ligand and receptor.

Example 4: Effects of COS cell-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

Because Ang-1-FD-Fc-FD is a mutant molecule derived from the agonist angiopoietin-1 and Ang-2-FD-Fc-FD is a mutant molecule derived from the antagonist angiopoietin-2, we wanted to determine whether or not these two mutant molecules would retain the activity associated with the parent molecule from which it was derived.

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Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA <u>80</u>:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

(A) Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with either 0.1 μg/ml, 0.2 μg/ml, or 0.8 μg/ml Ang1* or Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 9).

(B) Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.4 μ g/ml of the Tie-2 agonist Ang1* and 1 μ g/ml, 2 μ g/ml, 4 μ g/ml. 6 μ g/ ml, or 8 μ g/ml of Ang-2-FD-Fc-FD. As shown in Figure 10, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

- (C) Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor

 phosphorylation in EAhy926 cells: To compare the blocking effects of the naturally occurring antagonist angiopoietin-2 with that of Ang-2-FD-Fc-FD, the same experiment described in (B) supra was performed, substituting angiopoietin-2 for Ang-2-FD-Fc-FD. The results of this experiment are shown in Figure 11. At a 20 fold molar excess, the angiopoietin-2 has not reduced the phosphorylation level to 50%. This result, coupled with the results described in (B) supra implies that Ang-2-FD-Fc-FD is a more potent inhibitor or Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.
- 20 (D) Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the naturally occurring Tie-2 agonist angiopoietin-1 and 1 μg/ml, 2 μg/ml, 4 μg/ml. 6 μg/ ml, or 8 μg/ml of Ang-2-FD-Fc-FD. The results of this experiment, shown in Figure 12, show that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10 and described in (B) *supra*.
- (E) Ability of angiopoietin-2 to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the angiopoietin-1 and 1 μg/ml, 2 μg/ml, 4 μg/ml,

 $6~\mu g/$ ml, or $8~\mu g/$ ml of angiopoietin-2. The results of this experiment, shown in Figure 13, show that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11 and described in (C) *supra*.

Example 5: Construction of Ang-1-FD-Fc-FD CHO cell expression vector pRG763/Ang-1-FD-Fc-FD.

The pRG763/Ang-1-FD-Fc-FD CHO cell expression vector was constructed by isolating from the plasmid pCDNA3.1/Ang1-FD-Fc-FD a 2115 base pair EcoRI - NotI fragment containing Ang1-FD-Fc-FD and ligating this fragment into pRG763 vector digested with EcoRI and NotI. A large scale (2L) culture of E. coli DH10B cells carrying the pRG763/Ang-1-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was verified by digestion of aliquots with NcoI and HincII restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

Example 6: Expression of Ang-1-FD-Fc-FD in CHO cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of pRG763/Ang-1-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10%

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FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *infra*.

Example 7: Construction of Ang-2-FD-Fc-FD CHO cell expression vector pRG763/Ang-2-FD-Fc-FD.

The plasmid pRG763/Ang-2-FD-Fc-FD was constructed by isolating from the plasmid pCDNA3.1/Ang-2-FD-Fc-FD a 2097 base pair EcoRI - NotI fragment containing Ang-2-FD-Fc-FD and ligating this fragment into the pRG763 vector digested with EcoRI and NotI. A large scale (1L) culture of E. coli DH10B cells carrying the pRG763/Ang-2-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of plasmid DNA with NcoI and Ppu10I restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

25 <u>Example 8: Expression of Ang-2-FD-Fc-FD in CHO cells.</u>

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of pRG763/Ang-2-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after

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adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles purified as described infra.

10 Example 9: Characterization of stable CHO clone-derived Ang-1-FD-Fc-FD protein.

Molecular Weight Analysis: The predicted molecular weight for stable CHO clone-derived Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology

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Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. The results of this analysis show that the dimeric protein appears to be approximately 173.9kD and the presence of a single peak implies that the protein solution is homogenous.

Expression level of Ang-1-FD-Fc-FD in stable CHO clones: CHO cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-1-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of 2-3 pg/cell/day.

Purification of Ang-1-FD-Fc-FD protein derived from stable CHO clone supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). The CHO cell supernatant was purified as described *supra* and the purified ANG-1-FD-Fc-FD protein was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of stable CHO clone-derived Ang-1-FD-Fc-FD protein: Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal

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sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

5 Example 10: Characterization of stable CHO clone-derived Ang-2-FD-Fc-FD protein.

Molecular Weight Analysis: As described for stable CHO clone-derived Ang-1-FD-Fc-FD supra, the predicted molecular weight for stable CHO clone-derived Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT). The monomeric form of Ang-2-FD-Fc-FD has a predicted weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104. SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis confirmed the molecular weight (176.6kD) and revealed that the stable CHO clonederived Ang-2-FD-Fc-FD protein, like stable CHO clone-derived Ang-1-FD-Fc-FD, exists as a homogeneous species.

Expression level of Ang-2-FD-Fc-FD derived from stable CHO clones: CHO cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-2-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of approximately 1-2 pg/cell/day.

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Purification of stable CHO clone-derived Ang-2-FD-Fc-FD from cell supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The CHO cell supernatant was purified as described for stable CHO clone-derived Ang-1-FD-Fc-FD supra and was used in the studies described infra to further characterize this protein.

N-terminal sequencing of stable CHO clone-derived Ang-2-FD-Fc-FD protein: Purified stable CHO clone-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Asp-X-Ala-Glu-Val, wherein X is Cys. This sequence can be found at amino acids 17-21 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Example 11: Effects of stable CHO clone-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA <u>80</u>:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

(A) Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with 0.4 μ g/ml Ang1* or 0.2 μ g/ml or 0.4 μ g/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that or stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 17).

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(B) Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.2 μ g/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 μ g/ml, 4 μ g/ml, 8 μ g/ml or 16 μ g/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. As shown in Figure 18, Ang-2-FD-Fc-FD is able to block stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

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Ephrin ligands:

In previous experiments (Davis et al., 1994, Science, 266:816-819; Gale et al., 1996, Neuron 17:9-19, Gale and Yancopoulos, 1997, Cell Tissue Research 290:227-241), soluble, unclustered Ephrin-B1-Fc and Ephrin-B2-Fc, which dimerize at their respective Fc domains and therefore are dimeric with respect to either the Ephrin-B1 or Ephrin-B2 ectodomain, failed to induce EphB2 receptor phosphorylation. However, when either molecule was multimerized by pre-clustering with an anti-Fc antibody, they became potent agonists for the EphB2 receptor, as judged by tyrosine phosphorylation of the EphB2 receptor in a COS cell reporter assay. Because multimerization of both Ephrin-B1 and Ephrin-B2 appears to be necessary for induction of receptor phosphorylation, we theorized that a molecule that contained tandem repeats of either Ephrin-B1 or Ephrin-B2 ectodomains fused to an Fc domain, which would be dimeric with respect to the Fc domain but which would be tetrameric with respect to Ephrin ectodomains, might be sufficiently clustered to induce receptor phosphorylation. To test this hypothesis, the following DNA constructs were constructed, recombinant proteins produced, and reporter assays performed.

PCT/US99/30900 WO 00/37642

Construction of tandem Ephrin ectodomain/Fc domain nucleic acid molecules.

All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into either the mammalian expression pJFE14 (Ephrin-B1-Ephrin-B1-Fc) or pMT21 (Ephrin-B2-Ephrin-B2-Fc), each with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5' end to promote protein translation. The bridging sequences described infra were 15 introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a wery critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

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Example 12: Construction of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules.

(A) Ephrin-B1-Ephrin-B1-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B1 (Davis et al., ibid.), which corresponds to nucleotides 1-711 of Figure 14A-Figure 14B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 712-720 of Figure 14B), followed by a second copy of the ectodomain of Ephrin-B1 (corresponding to nucleotides 721-1344 of Figure 14B-Figure 14D), except that in this copy of the Ephrin-B1 ectodomain the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1345-1353 of Figure 14D),

followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1354-2049 of Figure 14D-Figure 14E).

(B) Ephrin-B2-Ephrin-B2-Fc: The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E).

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As with the angiopoietin nucleic acid molecules described *supra*, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

20 Example 13: Expression of tandem Ephrin recombinant proteins in COS cells.

COS cells were transiently transfected with either the Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules described *supra* using standard transfection techniques known in the art. Two days subsequent to transfection, the growth medium (DMEM supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM glutamine, and 10% calf serum) was aspirated and replaced with serum-free medium (DMEM supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM glutamine). Cell were grown for an additional three days and then the serum-free medium containing the recombinant proteins was collected. Recombinant protein concentration was determined by performing dot blots and comparing the

signal obtained to a standard curve. Once approximate protein concentrations were determined, the Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc recombinant proteins were used in the cell reporter assays described infra.

Example 14: Characterization of the COS cell-derived tandem Ephrin ectodomain/Fc domain recombinant proteins.

Reporter Assay: COS cells, which endogenously express the Eph family receptor EphB2 (Gale et al., 1996, Neuron 17:9-19), were used in reporter assays to evaluate the ability of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc to induce receptor phosphorylation. The assays were performed as previously described (Davis et al., ibid.; Gale et al., ibid.). Briefly, COS cells were grown to 80-90% confluency in standard growth 15 medium described supra. After growth, the medium was aspirated, and replaced with serum-free medium (described supra) for 1-2 hours prior to treatment with either Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc recombinant protein. The cells were stimulated with 500 ng/ml Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc for 30 minutes at 37°C, with or without affinity purified human IgG1 Fc-specific goat anti-human antibody (Jackson Immunoresearch, West Grove, PA) at a final concentration of 17 µg/ml. This antibody is capable of clustering the Fc tagged fusion. Subsequent to treatment, the COS cells were harvested and cell lysates were prepared as described in Davis, et al. and Gale, et al., supra. The EphB2 receptor protein was immunoprecipitated from the cell lysates using an anti-EphB2 antisera (Henkemeyer et al., 1994, Oncogene 2:1001-1014). Immunoprecipitates were resolved by standard SDS PAGE and transferred to PVDF membranes (Millipore) for western blot analysis. The membranes were probed with either anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology Institute, Lake Placid, NY) or anti-EphB2 antibodies (Henkemeyer, et al., ibid.) to determine the extent of EphB2

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phosphorylation and the relative quantities of EphB2 in the experimental conditions described *supra*.

Results: Both Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc were shown to be approximately as active as anti-Fc antibody-clustered Ephrin-B1-Fc in their ability to induce EphB2 receptor phosphorylation in the COS cell reporter assay. Furthermore, if either of the proteins were further clustered with the goat anti-human Fc antibody, they became even more potent in their ability to induce EphB2 receptor phosphorylation. Figure 16 shows the results of this phosphorylation assay.

Example 15: Construction of Ephrin-B2-Ephrin-B2-Fc CHO expression vector.

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The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 20 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E). This molecule was subcloned into the HindIII and NotI 25 polylinker sites in the expression vector pRG763 and was designated pRG763-m(Ephrin-B2)2-Fc. As with the angiopoietin nucleic acid molecules described supra, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains. 30

Example 16: Expression of Ephrin-B2-Ephrin-B2-Fc in CHO-K1 (E1A) cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 106 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal

Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μg of pRG763-m(Ephrin-B2)2-Fc using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10%

FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described supra.

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

- The nucleic acid of claim 1, wherein the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand.
- 3. The nucleic acid of claim 1, wherein the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit.
- 4. The nucleic acid of claim 2, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.
- 5. The nucleic acid of claim 3, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.
- 6. The nucleic acid of claim 2, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.
- 7. The nucleic acid of claim 4, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

8. The nucleic acid of claim 2, wherein the ligand is selected from the group consisting of the EPH family of ligands.

- 9. The nucleic acid of claim 4, wherein the ligand is selected from the group consisting of the EPH family of ligands.
- 10. The nucleic acid of claims 1 through 9, wherein the multimerizing component comprises an immunoglobulin derived domain.
- 11. The nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
- 12. A fusion polypeptide encoded by the nucleic acid molecule of claims 1 through 11.
- 13. A composition comprising a multimer of the fusion polypeptide of claim 12.
 - 14. The composition of claim 13, wherein the multimer is a dimer.
- 15. A vector which comprises the nucleic acid molecule of claims 1 through 11.
- 16. An expression vector comprising a nucleic acid molecule of claims 1 through 11, wherein the nucleic acid molecule is operatively linked to an expression control sequence.
- 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.

18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

- 19. The host-vector system of claim 17, wherein the suitable host cell is <u>E. coli.</u>
- 20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.
- 21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.
- 22. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 17 through 21, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.
- 23. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component.
- 24. The nucleic acid of claim 23, wherein the receptor binding domains are fused contiguously.
- 25. The nucleic acid of claim 23, wherein the ligand is not a member of the EPH family of ligands.
- 26. The nucleic acid of claim 24, wherein the ligand is not a member of

the EPH family of ligands.

27. The nucleic acid of claim 23, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

- 28. The nucleic acid of claim 24, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.
- 29. The nucleic acid of claims 23 through 28, wherein the multimerizing component comprises an immunoglobulin derived domain.
- 30. The nucleic acid molecule of claim 29, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
- 31. A fusion polypeptide encoded by the nucleic acid molecule of claims 23 through 30.
- 32. A composition comprising a multimer of the fusion polypeptide of claim 31.
- 33. The composition of claim 32, wherein the multimer is a dimer.
- 34. A vector which comprises the nucleic acid molecule of claims 23 through 30.
- 35. An expression vector comprising a nucleic acid molecule of claims 23 through 30, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

36. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 35, in a suitable host cell.

- 37. The host-vector system of claim 36, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.
- 38. The host-vector system of claim 36, wherein the suitable host cell is <u>E. coli</u>.
- 39. The host-vector system of claim 36, wherein the suitable host cell is a COS cell.
- 40. The host-vector system of claim 36, wherein the suitable host cell is a CHO cell.
- 41. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 36 through 40, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

1/42 Figure 1A

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2/42 Figure 1B

460	470	480 490
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ATC TTA CAC GGT GCT	GAT TITC AGC ACT	AAA GAT GCT GAT AAT GAC
Ile Leu His Gly Ala	Asp Phe Ser Thr	Lys Asp Ala Asp Asn Asp>
b b b ANGI	FIRRINOGEN_TIVE	DOMAIN_bbb>
2	TIDKINOGEM-DIKE	DOMAIN_BBBS
500 510	520	530 540
* * *	* *	530 540
AAC TGT ATG TGC AAA	שביי פרר בייר איירי	TTA ACA GGA GGA TGG TGG
Asn Cvs Met Cvs Lvs	.Cvc Ala Lou Mot	Leu Thr Gly Gly Trp Trp>
b b b ANGI	FIRPINOCEN_I TVE	DOMAIN_bbb>
	TENTHOGEN-DIKE	DOMAIN_BBB>
550	560	570 580
* * *	* *	* * * *
TTT GAT GCT TGT GGC	CCC TCC AAT CTA	AAT GGA ATG TTC TAT ACT
Phe Asp Ala Cvs Glv	Pro Ser Asn Leu	Asn Gly Met Phe Tyr Thr>
b b b ANGI	FIRRINGEN-LIKE	DOMAIN_bbb>
, .	- ADMINOGINE-DIKE	DOTAIN_BBS
590 600	610	620 630
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GCG GGA CAA AAC CAT	GGA AAA CTG AAT	GGG ATA AAG TGG CAC TAC
Ala Gly Gln Asn His	Gly Lys Leu Asn	Gly Ile Lys Trp His Tyr>
bbb_ANG1	FIBRINOGEN-LIKE	DOMAIN_bbb>
640	650	660 670
* * *	* *	
TTC AAA GGG CCC AGT	TAC TCC TTA CGT	TCC ACA ACT ATG ATG ATT
Phe Lys Gly Pro Ser	Tyr Ser Leu Arg	Ser Thr Thr Met Met Iles
bbANG1	FIRRINGEN_LIKE	DOMATIN b. b. b.
		DOMAIN_DD D D >
		DOFATN_BBS
680 690	700	710 720
680 690 * *	700	710 720 * * * *
680 690 * * * * * * * * * * * * * * * * * * *	700 * * GGC CCC GCG CCT	
680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe	700 GGC CCC GCG CCT	710 720 * * * *
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680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe	700 GGC CCC GCG CCT	710 720 * * TTT AGA GAC TGT GCA GAT
680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe	700 GGC CCC GCG CCT	710 720 * * ** ** ** ** ** ** ** ** ** ** ** **
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680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI	710 720 * * * * TTT AGA GAC TGT GCA GAT > Phe Arg Asp Cys Ala Asp>ANG1 FIBRINOGEN>
680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe	700 GGC CCC GCG CCT	710 720 * * TTT AGA GAC TGT GCA GAT > Phe Arg Asp Cys Ala Asp>
680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740	710 720 * * * * * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp> ANG1 FIBRINOGEN- 750 760 * * * *
680 690 * CGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO 730 * GTA TAT CAA GCT GGT	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT	710 720 * * * * * * * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp> ANG1 FIBRINOGEN> 750 760 * * * * GGA ATC TAC ACT ATT TAT
CGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT Phe Asn Lys Ser	710 720 * * * * * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp> ANG1 FIBRINOGEN> 750 760 * * * * GGA ATC TAC ACT ATT TAT Gly Ile Tyr Thr Ile Tyr>
CGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT	710 720 * * * * * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp> ANG1 FIBRINOGEN> 750 760 * * * * GGA ATC TAC ACT ATT TAT Gly Ile Tyr Thr Ile Tyr>
CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe ANG1 FIBRINO 730 GTA TAT CAA GCT GGT Val Tyr Gln Ala Gly d_d_d_ANG1	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE	710 720 * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp>ANG1 FIBRINOGEN> 750 760 * GGA ATC TAC ACT ATT TAT Gly Ile Tyr Thr Ile Tyr> DOMAIN_dddd>
CGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT Phe Asn Lys Ser	710 720 * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp>ANG1 FIBRINOGEN> 750 760 * * * * * * * * * * * * * * * * * * *
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CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe ANG1 FIBRINO 730 * GTA TAT CAA GCT GGT Val Tyr Gln Ala Gly d_d_d_ANG1 770 780 ATT AAT AAT ATG CCA Ile Asn Asn Met Pro	700 GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 * TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE 790 GAA CCC AAA AAG Glu Pro Lys Lys	710 720 * * * * * * * * * * * * * * * * * * *
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CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe ANG1 FIBRINO 730 * GTA TAT CAA GCT GGT Val Tyr Gln Ala Gly d_d_d_ANG1 770 780 ATT AAT AAT ATG CCA Ile Asn Asn Met Pro	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 * TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE 790 GAA CCC AAA AAG Glu Pro Lys Lys FIBRINOGEN-LIKE	710 720 * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp>ANG1 FIBRINOGEN> 750 760 * * * * * * * * * * * * * * * * * * *
CGA CCT TTA GAT TTT Arg Pro Leu Asp Phe ANG1 FIBRINO 730 * GTA TAT CAA GCT GGT Val Tyr Gln Ala Gly d_d_d_ANG1 770 780 * ATT AAT AAT ATG CCA Ile Asn Asn Met Pro d_d_d_ANG1	700 GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 * TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE 790 GAA CCC AAA AAG Glu Pro Lys Lys	710 720 * * * * * * * * * * * * * * * * * * *
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GGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO 730 GTA TAT CAA GCT GGT Val Tyr Gln Ala GlydddANG1 770 780 ATT AAT AAT ATG CCA Ile Asn Asn Met ProddANG1 820 GTC AAT GGG GGA GGT	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE 790 GAA CCC AAA AAG Glu Pro Lys Lys FIBRINOGEN-LIKE 830 TGG ACT GTA ATA	710 720 * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp>ANG1 FIBRINOGEN> 750 760 * * * * * * * * * * * * * * * * * * *
GGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO 730 GTA TAT CAA GCT GGT Val Tyr Gln Ala GlydddANG1 770 780 ATT AAT AAT ATG CCA Ile Asn Asn Met ProddANG1 820 GTC AAT GGG GGA GGT Val Asn Gly Gly Gly	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE 790 GAA CCC AAA AAG Glu Pro Lys Lys FIBRINOGEN-LIKE 830 TGG ACT GTA ATA Trp Thr Val Ile	710 720 * TTT AGA GAC TGT GCA GAT Phe Arg Asp Cys Ala Asp>ANG1 FIBRINOGEN> 750 760 * * * * * * * * * * * * * * * * * * *
GGA CCT TTA GAT TTT Arg Pro Leu Asp PheANG1 FIBRINO 730 GTA TAT CAA GCT GGT Val Tyr Gln Ala GlydddANG1 770 780 ATT AAT AAT ATG CCA Ile Asn Asn Met ProddANG1 820 GTC AAT GGG GGA GGT Val Asn Gly Gly Gly	GGC CCC GCG CCT Gly Pro Ala Pro GPAP BRI 740 TTT AAT AAA AGT Phe Asn Lys Ser FIBRINOGEN-LIKE 790 GAA CCC AAA AAG Glu Pro Lys Lys FIBRINOGEN-LIKE 830 TGG ACT GTA ATA Trp Thr Val Ile	710 720 * * * * * * * * * * * * * * * * * * *

3/42 Figure 1C

860	870		880		890	900
X C C C C C C C C C	* *	*	*	*	*	* *
AGT CTA GAT	Pho Cla	AGA GGC	TGG AAG	GAA TAT	r AAA ATG	GGT TTT
Ser Leu Asp	d ANGI	ETERTNO	TIP LYS	GIU Ty	Lys Met	Gly Phe>
	uANGI	FIBRINO	PEN-LIKE	DOMAIN_	_aa	dd>
. 9	10	920		930	9	40
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GGA AAT CCC	TCC GGT	GAA TAT	TGG CTG	GGG AAT	GAG TTT	ATT TTT
Gly Asn Pro	Ser Gly	Glu Tyr	Trp Leu	Gly Asi	ı Glu Phe	Ile Phe>
dd	dANG1	FIBRINO	SEN-LIKE	DOMAIN_	_dd	dd>
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GAC TGG GAA	GGG AAC	CGA GCC	TAT TCA	CAG TAT	GAC AGA	TTC CAC
Asp Trp Glu	Gly Asn	Arg Ala	Tyr Ser	Gln Ty	Asp Ara	Phe His>
dd	dANG1	FIBRINO	GEN-LIKE	DOMAIN	_dd	d
1040	1050		1060	. 1	L070	1080
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ATA GGA AAT	GAA AAG	CAA AAC	TAT AGG	TTG TAT	AAA ATT. ?	GGT CAC
Ile Gly Asn	d and	GIN ASN	Tyr Arg	Leu Ty	Leu Lys	Gly His>
	uMUI	ETRITIO	PEM-DIVE	DOMATH_	_aa	a>
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ACT GGG ACA	GCA GGA	AAA CAG	AGC AGC	CTG ATC	TTA CAC	GGT GCT
Thr Gly Thr	· Ala Gly	Lys Gln	Ser Ser	Leu Ile	Leu His	Glv Ala>
dd	_dANG1	FIBRINO	GEN-LIKE	DOMAIN	_dd	dd>
1130	1140	_	1150	. 1	1160	1170
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GAT TTC AGO Asp Phe Ser	The Type	Acr Ala	GAT AAT	GAC AAC	TGT ATG	TGC AAA
4 4	. IIII LIYS	ASP AIG	ASP ASH	MSD ASI	1 Cys Met	Cys Lys>
	d ANGI	FIRRING				
	dang1	FIBRINO	35M-TIVE	DOMAIN		aa>
. 11	_dang1 .80	1190		1200		·:
11	_dANG1					10
* TGT GCC CTC	_dANG1 .80 * * C ATG TTA	1190 * ACA GGA	* GGA TGG	1200 * TGG TT	12 * F GAT GCT	10 * *
* TGT GCC CTC Cys Ala Leu	_dANG1 .80 * * C ATG TTA 1 Met Leu	1190 ACA GGA Thr Gly	GGA TGG	1200 * TGG TT	12 * F GAT GCT P Asp Ala	10 * TGT GGC Cys Gly>
* TGT GCC CTC	_dANG1 .80 * * C ATG TTA 1 Met Leu	1190 ACA GGA Thr Gly	GGA TGG	1200 * TGG TT	12 * F GAT GCT P Asp Ala	10 * *
TGT GCC CTC Cys Ala Leu dd	_dANG1 .80 * * : ATG TTA : Met Leu _dANG1	1190 ACA GGA Thr Gly FIBRINO	* GGA TGG Gly Trp GEN-LIKE	TGG TT Trp Pho DOMAIN	12 F GAT GCT Asp Ala	TGT GGC Cys Gly>
* TGT GCC CTC Cys Ala Leu	_dANG1 .80 * * C ATG TTA 1 Met Leu	1190 ACA GGA Thr Gly FIBRINO	GGA TGG	TGG TT Trp Pho DOMAIN	12 * F GAT GCT P Asp Ala	10 * TGT GGC Cys Gly>
TGT GCC CTC Cys Ala Leudd 1220 *	_dANG1 .80 . ATG TTA 1 Met Leu _dANG1 	1190 ACA GGA Thr Gly FIBRINO	GGA TGG Gly Trp GEN-LIKE 1240	TGG TT Trp Pho DOMAIN	T GAT GCT Asp Ala d_d_d 1250	TGT GGC Cys Gly> dd>
TGT GCC CTC Cys Ala Leudd1220	.dANG1 .80 .t ATG TTA . Met Leu .dANG1 .1230 .t CTA AAT	1190 ACA GGA Thr Gly FIBRINO	GGA TGG Gly Trp GEN-LIKE 1240 * TTC TAT	TGG TT' TTP Pho DOMAIN	T GAT GCT ASP Ala d_d_d 1250 * G GGA CAP	TGT GGC Cys Gly> dd>
TGT GCC CTC Cys Ala Leudd 1220 * CCC TCC AAT Pro Ser Ass	.dANG1 .80 .t ATG TTA .t Met Leu .dANG1 .t ANG1 .t CTA AAT	1190 ACA GGA Thr Gly FIBRINO GGA ATG Gly Met	GGA TGG Gly Trp GEN-LIKE 1240 * TTC TAT Phe Tyr	TGG TT' Trp Pho DOMAIN ACT GCC Thr Alc	T GAT GCT ASP Ala d_d_d 1250 * G GGA CAP G Gly Glr	TGT GGC Cys Gly> dd>

4/42 Figure 1D

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Gly	Lys	Leu	Asn	Gly	Ile	Lys	Trp	His	Tyr	Phe	Lys	Gly	Pro	Ser>
	dc	£	đ2	ANG1	FIBE	RINOC	GEN-I	LIKE	DOM	AIN_c	i	a c	1	d>
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Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu>
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GIU	e vai	. IIIE	Cys -	· var	val e =	vai	Asp	vai	ser	Hls	GLu	Asp	Pro	Glu>
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AAG	580 * ACA	AAG	* CCG	1590 * CGG	GAG	* GAG	CAG	* TAC	* : AAC	AGC	* ACG	TAC	* CGT	* GTG
AAG	580 * ACA	AAG	* CCG	1590 * CGG	GAG Glu	* GAG Glu	CAG	* TAC	* AAC Asn	AGC	* ACG	TAC	* CGT	* GTG
AAG Lys	580 * ACA Thr	AAG Lys	* CCG Pro	1590 CGG Arg	Glu	Glu	CAG Gln	* TAC Tyr	Asn	AGC Ser	* ACG Thr	Tyr	* CGT	* GTG Val>
AAG Lys	580 * ACA Thr	AAG Lys	* CCG Pro	1590 CGG Arg	Glu	Glu	CAG Gln	* TAC Tyr	Asn	AGC Ser	* ACG Thr	Tyr	* CGT	* GTG
AAG Lys	580 * ACA Thr	AAG Lys f	t CCG Pro	1590 CGG Arg	Glu fF	Glu C TA	CAG Gln	* TAC Tyr	Asn	AGC Ser f	* ACG Thr	Tyr f	* CGT Arg	* GTG Val>
AAG Lys	580 * ACA Thr	AAG Lys f	* CCG Pro	1590 CGG Arg	Glu fF	Glu	CAG Gln	* TAC Tyr	Asn	AGC Ser f	* ACG Thr	Tyr f	* CGT	* GTG Val>
AAG Lys	580 * ACA Thr f	AAG Lys f	t CCG Pro f	CGG Arg	Glu fF	Glu C TA	CAG Gln G [S	* TAC TYT PLIT	1650	AGC Ser f	ACG Thr	Туг f	* CGT Arg f	* GTG Val>
AAG Lys 	ACA Thr	AAG Lys f	CTC	CGG Arg	Glu fF	Glu C TA 640 * CTG	CAG Gln G [S	TAC TYT PLIT	1650 *	AGC Ser f	ACG Thr f	Tyr f16	* CGT Arg f	* GTG Val>

5/42 Figure 1E

1670	1680	1690	1700	1710
*	* *	* *.	* *	* *
GAG TAC AAG	G TGC AAG GTC	TCC AAC AA	A GCC CTC CCA GO	CC CCC ATC
Giu Tyr Lys	s Cys Lys Val	Ser Asn Lvs	Ala Leu Pro A'	a Pro Tlas
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Clu Lvc Th	ATC TCC AAA	GCC AAA GGC	G CAG CCC CGA G	AA CCA CAG
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GTG TAC ACC	CTG CCC CCA	TCC CGG GAT	GAG CTG ACC A	יי ביי
var Tyr Thi	r Leu Pro Pro	Ser Arg Ast	O. Glu Leu Thr Ja	e Aen Clas
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val Ser Let	Thr Cys Leu	Val Lys Gly	Phe Tyr Pro Se	er Asp Ile>
		C TAG [SPLIT	[]fff	_ff>
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Ala Val Glu	Tro Glu Ser	Asn Gly Glr	Pro Glu Asn As	AC TAC AAG
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ACC ACG CC	CCC GTG CTG	GAC TCC GAC	GGC TCC TTC T	CTC TAC
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f f	s Ser val Met	His Glu Ala	Leu His Asn H	is Tyr Thr>
		C TAG [SPLI]	r)ff	f>
2030	2040	2050	*	
*	* *	* *		
CAG AAG AG	C CTC TCC CTG	TCT CCG GGT	C AAA TGA	
Gln Lys Ser	r Leu Ser Leu	Ser Pro Gly	/ Lvs ***>	
ff	_fFC TAG [.S	PLIT]f_	_f>	

6/42 Figure 2A 20 ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala> a a a TRYPSIN SIGNAL SEQUENCE a_ AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC Arg Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly> _b__b__b__ang2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__ 110 120 . * ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC Ile Tyr Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala> b_b_ang2 fibrinogen-like domain #1__b_b_b_ 140 160 TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln> b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__b_b_b_> 200 210 * CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu> _b__b__b__ang2 fibrinogen-like domain #1___b__b__b__> 250 * * 240 260 * * TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly> _b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__> 280 * AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu> b b ang2 fibrinogen-like domain #1 b b b 330 AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu> b_b_b_ang2 fibrinogen-like domain #1__b_b_b_: 380 TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile> b_b_b_ang2 fibrinogen-like domain #1__b_b_ CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile> ___b__b__ang2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b___

7/42 Figure 2B

480 AGC CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp> __b_b_b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__b_b_b_> .500 510 520 AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp> __b_b_ang2 fibrinogen-like domain #1__b_b_b_> 3.2 TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro> _b__b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__b_ 590 620 610 CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr> _b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__> TGG AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile> _b__b__b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b__b__> 700 CGA CCA GCA GAT TIC GGG GGC CCC GCG CCT TTC AGA GAC TGT GCT Arg Pro Ala Asp Phe> ___ANG2 FIBRINO_ Gly Gly Pro Ala Pro> __GGPAP BRIDGE_ Phe Arg Asp Cys Ala> __ANG2 FIBRINO_ GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu> _d__d__d__ANG2 FIBRINOGEN-LIKE DOMAIN#2___d__d__d__> 790 780 ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met> _d__d__d_ANG2 FIBRINOGEN-LIKE DOMAIN#2___d__d__d__> 820 830 840 * . . . * GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT Glu Ala Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp> __d__d__d__ANG2 FIBRINOGEN-LIKE DOMAIN#2___d__d__d__>

8/42 Figure 2C

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Lys	Asp	Trp	Glu	Cly	Asn	Glu	Ala	Tyr	Ser	Leu	Tyr	Glu	His	Phe>
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9/42 Figure 2D

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10/42 Figure 2E

1670	1680	169	0	1700	1710
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AAG GAG TA	C AAG TGC AA	G GTC TCC	AAC AAA G	SCC CTC CCA	GCC CCC
ras Gin La	r Lys Cys Ly	s Val Ser	Asn Lys A	la Leu Pro	Ala Pro>
rr_	_ff	_fFC TAG	ff_	ff	
. 1	.720	1720	1740		
*	* *	1730	1740	175	0
ATC GAG AA	A ACC ATC TC	7 AAA GCC	3 3 A CCC C		* * *
Ile Glu Ly	s Thr Ile Se	r Ivs Ala	Ive Cly C	In Dro Ara	GAA CCA
ff	_ff	_f_ FC TAC	rys Gry G	f f f	Gin bro>
1760	1770	178	0	1790	1800
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CAG GTG TA	C ACC CTG CC	C CCA TCC	CGG GAT C	SAG CTG ACC	AAG AAC
Gin vai Ty	r Thr Leu Pr	Pro Ser	Arg Asp G	lu Leu Thr	Ive Aens
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Gln Val Se	r Leu Thr Cy	s Leu Val	ING CIV F	he Tir Pro	AGC GAC
ff	_fff	_fFC_TAG	f f	f f f	Ser Asp>
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1850	1860	. % 187	0	1880	1890
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ATC GCC GT	G GAG TGG GA	G AGC AAT	GGG CAG C	CG GAG AAC	AAC TAC
TIE VIE A	l Glu Trp Gl	Ser Asn	Gly Gln I	ro Glu Asn	Asn Tyr>
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AAG ACC AC	G CCT CCC GT	G CTG GAC	TCC GAC C	GC TCC TTC	שער כער
Lys Thr Th	r Pro Pro Val	l Leu Asp	Ser Asp C	ly Ser Phe	Phe Leus
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f f	_fff	r ASP Lys f FC TAC	Ser Arg 1	rp Gin Gin	Gly Asn>
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Val Phe Se	er Cys Ser Va	l Met His	Glu Ala I	eu His Asn	His Tvr>
tt	_ff	_fFC TAG	ff_	ff	
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2030	2040	205	• •	2060	
ACG CAG AA	G AGC CTC TC	רשכ שכש		A A	
Thr Gln Lv	s Ser Leu Se	r Leu Ser	Pro Gly I	MA IGA	
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11/42 Figure 3A

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Arg	Asp	Cys	Ala	Asp	Val	Tyr	Gln	Ala	GIA	Pne	Asn	rys.	Ser	Gly>
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TTT	TGC	AAT	ATG	GAT	GTC	AAT	GGG	GGA	GGT	TGG	ACT	GTA	ATA	CAA
Phe	Cys	Asn	Met	Asp	Val	Asn	Gly	Gly	Gly	Trp	Thr	Val	Ile	Gln>
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His	Arc	Glu	. Asr	Gly	Ser	Leu	AST	Pne	GIL	L ATG	r GTA	n Trp	ь Г	Glu>
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ጥል፣	r . A A .	MTA A	s GG	r. data	r GGZ	LAA A	ccc	TCC	GG1	GAZ	TA	TG0	CT	GGG
Tv	r Iv	s Mei	c Gly	, Phe	Gl	✓ Asr	Pro	o Sei	r Gly	, Gl	туі	· Trp	Lei	ı Gly>
-3.	ь ь	b	b	ANG	FI	BRING	GEN	-LIK	E DON	MIN	_b	_b	_b	_b>
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AA	T GA	G TT	T AT	TT TT	r GC	CAT	r AC	C AG	T CA	G AG	G CA	G TA	TA	G CTA
As	n Gl	u Ph	e Il	e Ph	e Al	a Il	e Th	r Se	r Gl	n Ar	g Gl	n Ty:	r Me	t Leu>
<u>-</u>	_b	_b	_p	_ANG	1 FI	BRIN	OGEN	-LIK	E DO	MAIN	_b	b	_p	_b <u>·</u> ->
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	320		_	33	0 .			340		*	350			. 30U *
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AG	A AL	പരവ	11 T.A	n wr	t De	ים דים דים מ	D GJ	u 61	v As	n Ar	g Al	a Tv	r Se	r Gln>
AI	A TI	.e Gi	b b	ANG	1 FI	BRIN	OGEN	-LIK	E DO	MAIN	_b	_b	_b_	_b>
	~	~									-			
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T	T G	C AC	TT A	C CA	C A	'A GG	A A	AT GA	AA AA	G CA	A Á	C TA	A T.	G TTG
T	r As	A q	g Pi	ne Hi	s Il	le Gl	y As	sn Gl	u Ly	rs Gl	n As	n Ty	r Ai	g Leu>
	b	b	b	ANC	31 F	BRIN	10GE1	1-LI	E DC	/IAM	I_p	b	_¤_	_b>

12/42 Figure 3B 430

410	420		430		440	450
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TAT TTA AAA GGT						
Tyr Leu Lys Gly	His	Thr Gly	Thr Ala	Gly Lys	Gln Ser	Ser Leu>
bbb	MG1	FIBRINO	EN-LIKE	DOMAIN_	bb	ob>
460						
460	_	470		480	49	90
100 mm1 010 com	*	*	*	*	*	* *
ATC TTA CAC GGT	GCT	GAT TTC	AGC ACT	AAA GAT	GCT GAT	AAT GAC
Ile Leu His Gly	Ala .	Asp Phe	Ser Thr	Lys Asp	Ala Asp	Asn Asp>
bbb	TMGT .	FIBRINO	SEN-LIKE	DOMAIN_I	ob1	ob>
500	510		520		- 2.0	
* *	*	*	520		530	540
AAC TGT ATG TGC	222	יי פרר	בתיכי אתיכי			maa
Asn Cys Met Cys	Tye	Cve Ala	Lou Mot	TIA ACA	GGA GGA	TGG TGG
bb <i>b</i>	MC1	CJS AIG FIRRIMO	Ded Met	DONATH I	GIA GIA	TIP TIP>
	_,	· zbkino	PH-PIKE	DOMAIN_I	JB	o>
550		560		570	51	30
, * *	*	*	*	*	*	* *
TTT GAT GCT TGT	GGC	CCC TCC	AAT CTA	AAT GGA	ል ጥር ጥጥር	ጥልጥ ልርጥ
Phe Asp Ala Cys	Gly	Pro Ser	Asn Leu	Asn Glv	Met Phe	Tyr Thre
bbp	NG1	FIBRINO	EN-LIKE	DOMAIN I	o b i) b >
•					 -	
590	600		610	(520	630
*	*	*	*	*	*	* *
GCG GGA CAA AAC	CAT	GGA AAA	CTG AAT	GGG ATA	AAG TGG	CAC TAC
Ala Gly Gln Asn	His (Gly Lvs	Leu Asn	Gly Ile	LVS Tro	His Turs
b b b b	MC2					
	7497	LIBKINO	SEN-LIKE	DOMAIN_	obl	bb
bbbA	7/61		SEN-LIKE	DOMAIN_	ob1	ob>
640	MGI	650	SEN-LIKE	·660		ob> 70
640 * *	*	650 *	*	·660 *	6	70 * *
640 * * TTC AAA GGG CCA	* AGT	650 * TAC TCC	* TTA CGT	TCC ACA	6°	70 * * ATG ATT
* * * TTC AAA GGG CCA Phe Lys Gly Pro	* AGT	650 * TAC TCC Tyr Ser	* TTA CGT Leu Arg	* TCC ACA Ser Thr	6° ACT ATG	70 * * ATG ATT Met lle>
640 * * TTC AAA GGG CCA	* AGT	650 * TAC TCC Tyr Ser	* TTA CGT Leu Arg	* TCC ACA Ser Thr	6° ACT ATG	70 * * ATG ATT Met lle>
* * * TTC AAA GGG CCA Phe Lys Gly Probbi	AGT Ser	650 * TAC TCC Tyr Ser	* TTA CGT Leu Arg SEN-LIKE	*TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met	70 * ATG ATT Met Ile> bb>
* * * TTC AAA GGG CCA Phe Lys Gly Pro	* AGT	650 * TAC TCC Tyr Ser	* TTA CGT Leu Arg	*TCC ACA Ser Thr DOMAIN_	6° ACT ATG	70 * * ATG ATT Met lle>
TTC AAA GGG CCA Phe Lys Gly Probbr	AGT Ser NG1	650 * TAC TCC Tyr Ser FIBRINOX	TTA CGT Leu Arg SEN-LIKE 700	** TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met D	70 * * * ATG ATT Met Ile> bb> 720 * *
TTC AAA GGG CCA Phe Lys Gly Probbb 680 CGA CCT TTA GAT	AGT Ser NG1 690	650 * TAC TCC TYr Ser FIBRINOX * GGC CCG	TTA CGT Leu Arg SEN-LIKE 700	** TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met D	70 * * * ATG ATT Met Ile> bb> 720 * *
TTC AAA GGG CCA Phe Lys Gly Probbf 680 CGA CCT TTA GAT Arg Pro Leu Asp	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC TYr Ser FIBRINOX * GGC CCG	TTA CGT Leu Arg SEN-LIKE 700	** TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met D	70 * * * ATG ATT Met Ile> bb> 720 * *
TTC AAA GGG CCA Phe Lys Gly Probbb 680 CGA CCT TTA GAT	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC Tyr Ser FIBRINO * GGC CCG	TTA CGT Leu Arg SEN-LIKE 700 * GGC GAG	** TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met D	70 * * * ATG ATT Met Ile> bb> 720 * *
TTC AAA GGG CCA Phe Lys Gly Probbf 680 CGA CCT TTA GAT Arg Pro Leu Asp	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC Tyr Ser FIBRING GGC CCG	TTA CGT Leu Arg GEN-LIKE 700 * GGC GAG Gly>	** TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met D	70 * * * ATG ATT Met Ile> bb> 720 * *
TTC AAA GGG CCA Phe Lys Gly Probbf 680 CGA CCT TTA GAT Arg Pro Leu Asp	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC Tyr Ser FIBRING GGC CCG	TTA CGT Leu Arg SEN-LIKE 700 * GGC GAG Gly>	* TCC ACA Ser Thr DOMAIN_	ACT ATG Thr Met D	ATG ATT Met Ile> bb> 720 * GAC AAA
TTC AAA GGG CCA Phe Lys Gly Probbf 680 CGA CCT TTA GAT Arg Pro Leu Asp	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC Tyr Ser FIBRING GGC CCG	TTA CGT Leu Arg SEN-LIKE 700 * GGC GAG Gly>	TCC ACA Ser Thr DOMAIN_I * CCC AAA	ACT ATG Thr Met D	ATG ATT Met Ile> bb> 720 * GAC AAA
TTC AAA GGG CCA Phe Lys Gly Probbf 680 CGA CCT TTA GAT Arg Pro Leu Asp	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC Tyr Ser FIBRING GGC CCG	TTA CGT Leu Arg SEN-LIKE 700 * GGC GAG Gly>	TCC ACA Ser Thr DOMAIN_I * CCC AAA	ACT ATG Thr Met D	ATG ATT Met Ile> bb> 720 * GAC AAA
TTC AAA GGG CCA Phe Lys Gly Probbf 680 CGA CCT TTA GAT Arg Pro Leu Asp	AGT Ser ANG1 690 TTT Phe>	650 * TAC TCC Tyr Ser FIBRING GGC CCG	TTA CGT Leu Arg SEN-LIKE 700 * GGC GAG Gly>	TCC ACA Ser Thr DOMAIN_I * CCC AAA	ACT ATG Thr Met 710 TCT TGT Ser Cys	ATG ATT Met Ile> bb> 720 * GAC AAA
TTC AAA GGG CCA Phe Lys Gly Probbr 680 CGA CCT TTA GAT Arg Pro Leu AspANG1 FIBRINO_	AGT Ser NG1 690 TTTT Phe>	650 * TAC TCC TYP SET FIBRINO GGC CCG Gly Pro 740 *	TTA CGT Leu Arg GEN-LIKE 700 * GGC GAG Gly> Glu	TCC ACA SET THT DOMAIN_ CCC AAA Pro LysdF	ACT ATG Thr Met 710 TCT TGT Ser Cys TAG 7	70 ATG ATT Met Ile> bb> 720 * GAC AAA Asp Lys> dd> 60 *
TTC AAA GGG CCA Phe Lys Gly Probb 680 CGA CCT TTA GAT Arg Pro Leu AspANG1 FIBRINO_ 730 ACT CAC ACA TGC	AGT Ser NG1 690 TTTT Phe>	650 * TAC TCC TYP SET FIBRINO GGC CCG Gly Pro 740 * CCG TGC	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA	TCC ACA SET THT DOMAIN_ * CCC AAA Pro Lys df 750 * CCT GAA	ACT ATG Thr Met 710 TCT TGT Ser Cys TAG 7 CTC CTG	ATG ATT Met Ile> bb> 720 * GAC AAA Asp Lys> dd> 60 *
TTC AAA GGG CCA Phe Lys Gly Probbr 680 CGA CCT TTA GAT Arg Pro Leu AspANG1 FIBRINO_ 730 ACT CAC ACA TGC Thr His Thr Cys	AGT Ser NG1 690 TTTT Phe>	GGC CCG Gly Pro CCG TGC Pro Cys	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala	TCC ACA SET THT DOMAIN_ * CCC AAA Pro LysdF 750 * CCT GAA Pro Glu	ACT ATG Thr Met 710 TCT TGT Ser Cys TAG 7 CTC CTG Leu Leu	ATG ATT Met Ile> bb> 720 * GAC AAA Asp Lys> dd> 60 * GGG GGA Gly Gly>
TTC AAA GGG CCA Phe Lys Gly Probb 680 CGA CCT TTA GAT Arg Pro Leu AspANG1 FIBRINO_ 730 ACT CAC ACA TGC	AGT Ser NG1 690 TTTT Phe>	GGC CCG Gly Pro CCG TGC Pro Cys	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala	TCC ACA SET THT DOMAIN_ * CCC AAA Pro LysdF 750 * CCT GAA Pro Glu	ACT ATG Thr Met 710 TCT TGT Ser Cys TAG 7 CTC CTG Leu Leu	ATG ATT Met Ile> bb> 720 * GAC AAA Asp Lys> dd> 60 * GGG GGA Gly Gly>
TTC AAA GGG CCA Phe Lys Gly Probbr 680 CGA CCT TTA GAT Arg Pro Leu AspANG1 FIBRINO_ 730 ACT CAC ACA TGC Thr His Thr Cysdddc	AGT Ser ANG1 690 TTTT Phe>	GGC CCG Gly Pro CCG TGC Pro Cys	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala	TCC ACA SET THT DOMAIN_ CCC AAA Pro Lys d_f 750 * CCT GAA Pro Glu d_d_d	ACT ATG Thr Met TOT TGT Ser Cys TAG TCT CTG CTC CTG Leu Leu d d d	ATG ATT Met Ile> bb> 720 * GAC AAA Asp Lys> dd> 60 * GGG GGA Gly Gly>
TTC AAA GGG CCA Phe Lys Gly Probbr 680 CGA CCT TTA GAT Arg Pro Leu AspANG1 FIBRINO_ 730 ACT CAC ACA TGC Thr His Thr Cys	AGT Ser NG1 690 TTTT Phe>	GGC CCG Gly Pro CCG TGC Pro Cys	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala	TCC ACA SET THT DOMAIN_ CCC AAA Pro Lys d_f 750 * CCT GAA Pro Glu d_d_d	ACT ATG Thr Met 710 TCT TGT Ser Cys TAG 7 CTC CTG Leu Leu	ATG ATT Met Ile> bb> 720 * GAC AAA Asp Lys> dd> 60 * GGG GGA Gly Gly>
TTC AAA GGG CCA Phe Lys Gly Pro b b b 7 680 CGA CCT TTA GAT Arg Pro Leu Asp ANG1 FIBRINO 730 ACT CAC ACA TGC Thr His Thr Cys d d d c	AGT Ser NG1 690 TTT Phe>	GGC CCG Gly Pro CCG TGC Pro Cys L	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala TAG 790	TCC ACA Ser Thr DOMAIN_ * CCC AAA Pro Lys df 750 * CCT GAA Pro Glu dd *	ACT ATG Thr Met D	ATG ATT Met Ile> bb> 720 * GAC AAA ASP Lys> dd> 60 * GGG GGA Gly Gly> dd> 810 *
TTC AAA GGG CCA Phe Lys Gly Pro b b b 7 680 CGA CCT TTA GAT Arg Pro Leu Asp ANG1 FIBRINO ACT CAC ACA TGC Thr His Thr Cys d d d c 770 CCG TCA GTC TTC	AGT Ser NG1 690 TTT Phe> CCA Pro 1 d 780	650 * TAC TCC TYP Ser FIBRINO GGC CCG Gly Pro C GTGC Pro Cys CCG TGC Pro Cys TTC CCC	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala TAG 790 * CCA AAA	TCC ACA Ser Thr DOMAIN_ * CCC AAA Pro Lys d_f 750 * CCT GAA Pro Glu d_d_d * CCC AAG	ACT ATG Thr Met 10 10 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	ATG ATT Met Ile> b_b_> 720 * GAC AAA Asp Lys> d_d_d_> 60 * GGG GGA Gly Gly> d_d_d_> 810 * CTC ATG
TTC AAA GGG CCA Phe Lys Gly Pro b b b 7 680 CGA CCT TTA GAT Arg Pro Leu Asp ANG1 FIBRINO 730 ACT CAC ACA TGC Thr His Thr Cys d d d c	AGT Ser NG1 690 TTT Phe> CCA Pro i d 780 CTC Leu	GGC CCG Gly Pro CCG TGC Pro Cys TTC CCC Phe Pro	TTA CGT Leu Arg SEN-LIKE 700 GGC GAG Gly> Glu CCA GCA Pro Ala TAG 790 CCA AAA Pro Lys	TCC ACA Ser Thr DOMAIN_ * CCC AAA Pro Lys d_f 750 * CCT GAA Pro Glu d_d CCC AAG	ACT ATG Thr Met D	ATG ATT Met Ile> b_b_> 720 * GAC AAA Asp Lys> d_d_d_> 60 * GGG GGA Gly Gly> d_d_d_> 810 * CTC ATG Leu Met>

13/42 Figure 3C

					rrgu	re 3	C						
	*	820	*	:	830 · *			840		•	8	50	*
ATC TO	יר כפ	G ACC	CCT	GNG	CTC	A.C.A	TCC	CTC	CTC	CTC	CNC	CMC	N.C.C
Ile Se	er Ar	g Thr	Pro	Glu	Val	Thr	Cvs	Val	Val	Val	Asp	Val	Ser>
d_	d	_dc	đć		F(TAC	G	<u>. </u>	i(dc	ic	ر نب	i>
86)	٠.	870	٠	٠.	88	30			890 [,]			900
CAC G	A G	*. AC CCT	¢ GAG	CTC	*	መጥር	*	* TCC	ጥልሮ	C WC	CAC	*	* CMC
His G	lu As	sp Pro	Glu	Val	Lvs	Phe	Asn	Tro	Tvr	Val	Asp	Glv	Val>
d_	d	_d	dc	<u></u>	F(TAC	30	dc	1	dc	3 <u>.</u>		i>
		910		9	20	. *		.930			94	10 -	
C) C C	*	* *	*		*		*	*		*		*	*
Glu V	iG CA	TAA TAI Ls Asn	Ala	TAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC
d_	d_	_d	d c	lys 1	i F	Dys TAC	3 (d d	1	d d	d in	lyr	ASN>
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95	0 .		960			97	70		- :	980			990
ACC A	ר. רוב יחיו	AC CGT	GTC.	CTC	NCC.	CONC	Curc.	NOC	CITIC	OMC.		*	*
Ser T	hr Ty	yr Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	ASD>
d_	a_	a	ao	10	1F	TAC	3	d	a	d	ao	i	d>
			٠.	_						•			
	*	1000	*	-10	010			1020	:		10:	30	
TGG C	rg a	AT GGC	AAG	GAG	TAC	AAG	TGC	AAG	GTC	TCC	AAC	AAA	GCC ,
Trp L	eu A	sn Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala>
d_	d_	d	đ	i	dF	C TAC	G	d	d	d	d(i	d>
104	0 * .	*	1050			10	60	•	`, 1	070		. :	1080
CTC C	CA G	cc ccc	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA	GGG	CAG
Leu P	ro A	la Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln>
d_	d_	d	d	đ	dF	C TA	G	d	đ	d	d	đ	đ>
		1090		1	100			1110			11	20	
	*	*	*		*		*	*		*		*	*
CCC C	GA G	AA CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG
Pro A	rg G	lu Pro	Gln	_Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu>
u_	u_	a	.u	u	ur	C TA	G	.a	α	<u>-a</u>	.a	a	a>
113	o		1140			11	50	.:	1	160			1170
	*	*	*		*		*	i v 🛊		* v *	٠	*	*
CTG A	CC A	AG AAC ys Asn	CAG	GTC	AGC	CTG	ACC	TGC	CTC	GTC	AAA	GGC	TTC
d_	d_	d	d	d	dF	C TA	.G	d d	d	d	d d	d d	d >
,		•											
	* 1	1180		1	190		*	1200	•	*	12	10	*
TAT C	CC A	GC GAC	ATC	GCC	GTG	GAG	TGG	GAG	AGC	TAA C	GGG	CAG	CCG
Tyr F	ro S	er Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro>
a_	a_	d	<u>a</u>	a	.aF	C TA	.G	_d	_d	_d	_d	.d	_d>
122	0	•	123Ò		*	12	40		. 1	1250		*	1260
GAG. A	AC A	AC TAC	: AAG	ACC	ACG	CCI	ccc	GTO	CT	G GAC	TCC	GAC	GGC
Glu A	sn A	sn Tyr	Lys	Thr	Thr	Pro	Pro	o Val	Lei	ı Asp	Ser	Asp	Gly>
d_	d_	d	_d	d	.dF	C TA	.G	_d	_d	_d	_d	_d	_d>

14/42 Figure 3D

		127	70		12	80		:	1290			130	0	
	*		*	*		*		*	*		*		*	*
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														Trp>
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13	10			1320			133	30	٠.	1.	340		. 1	L350
010	C 2 C	000	*	GTC		*		*	- *		,* 	~~~	*	*
CAG .	CAG	GGG	AAC	Ual	Pho	TCA	CVC	TCC	GIG	Mot	UAT	Clu	Ala	Leu>
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		13	50		1.	370			1380			139	90	
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CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys>
Ġ	<u> </u>	d	d	d	d	dF(C TA	G	<u>ط</u> ــــــــب	d	d0	dc	i	d>
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14	0.0			1410	·		14	20		1	430			1440
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				TCT					AGA	GAC	TGT	GCA	GAT	GTA
_		_		Ser SC B								•		
		DIME	EK/ H	SC D	KIDG.	C (1V			2	» en	Circ	N 1 -	200	Val>
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		14	5 0 .	:	. 1	460			1470			14	80	
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TAT	CAA	GCT	GGI	TTT	AAT	AAA	AGT	GGA	ATC	TAC	ACT	ATT	TAT	TTA
														Ile>
	E	f	£	_ANG1	FIB	RINO	GEN-	LIKE	DOM	AIN_	£	£	£	£>
				· · .								:	:	
. 14	190			1500			15	10		1	520			1530
ידעע	ייי א א	· h πc	י רכז	ממבט נ	ccć	 תתת	7.70	_ 	י שאואט י	- W-C	י. יחאר	אתיכי	CAM	GTC
														Val>
ASII	F													f>
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	. *.		*	.*		*		*	. *		. *		*	• •
														AGT
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				1500		•					610	-		
. 1	280		*	15.90	,		16	500			610			1620
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	, •		*	•	•	•		♠,	•	•	*		*	*
														r GCC
Asn	Pro	Se	r G1	y Glu	туз	Tr	e Le	ı Gl	y Ası	ı Glı	ı Phe	e Ile	Phe	Ala:

15/42 Figure 3E

1670	1	1680			169	90		17	700		:	1710
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ATT ACC A												
ff_	f1	ANG1	FIBE	RINO	GEN-I	LIKE	DOMA	IN_	5f	E f	Met	Asp>
				• •	•			_				
	1720		17	730		. :	1740			175	0	
TGG GAA G	* CC 33C	CC2	000	* ~~~	mc s	*	*	010	*		*	*
Trp Glu G												
ff_	f/	ANG1	FIB	RINO	GEN-1	LIKE	DOMA	IN_	F1	E 1		£ >
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1760	. :	1770			178	30		1	790		:	1800
GGA AAT G	מגג גב	ת אם	220	mam.	N.C.C	*	*	mm s	*		*	*
Gly Asn G	lu Lvs	Gln	Asn	TVT	Ara	Tien	TAT	Len	TAZE	GGT	CAC	ACT
ff_	f	ANG1	FIB	RINO	GEN-1	LIKE	DOM	AIN_:	E1	E :	E	f >
• •		•						_			-	
•	1810		10	320		_ :	1830			184	10	
GGG ACA G	יי מכי	מממ	CAG	» «	N.C.C	T T T) W	mm s	*	COM	*	*
Gly Thr A	la Gly	Lvs	Gln	Ser	Ser	Leu	Ile	Leu	His	GU	Ala	GAT.
ff_	f	ANG1	FIB	RINO	GEN-	LIKE	DOM	AIN_	£:	E:	E	f>
•												
1850		1860	•		18	70		. 1	880			1890
TTC AGC A	מא אים רית א	ת מים	CCT	C 2 m	n nei	* C3C	*	mam	*	maa.	*	, * , mam
Phe Ser T	hr Lvs	ASD	Ala	Asp	Asn	ASD	AAC	Cve	Mot	TGC	AAA	TGT
ff_	f	ANG1	FIB	RINO	GEN-	LIKE	DOM	_NIA	f:	£	E	f >
								_	•	-		
•	1900		1:	910	٠.		1920			19	30	
GCC CTC A	מידים. מידים	מים ב	CCA	GCV	TCC	ancic. *	andra.	Cam	~ ~	mcm.	*	*
Ala Leu M	let Leu	Thr	Glv	Glv	Tro	Tro	Phe	Asp	Ala	CVS	GGC	Prox
ff_	f	ANG1	FIB	RINO	GEN-	LIKE	DOM	_NIA	f	f	£	£>
	٠.								•			
1940	•	1950			19	60	_	1	970		_	1980
TCC AAT C	TA AAT	GGA	ATG	TTC	TAT	ACT.	GCG ,	CCA	ממר)	Δ A C	Υ Cλπ	CCA
Ser Asn I												
ff_	f	ANG1	FIB	RINO	GEN-	LIKE	DOM	AIN_	£	£	£	f>
•	1000		_					•				
•	1990 *	*	2	000		*	2010		*	20	20	
AAA CTG A	AT GGG	ATA	AAG	TGG	CAC	TAC	TTC	AAA	GGG	CCA	AGT	TAC
Lys Leu A	Asn Gly	Ile	Lys	Trp	His	Tyr	Phe	Lys	Gly	Pro	Ser	Tyr>
ff_	f	ANG1	FIB	RINO	GÉN-	LIKE	DOM	AIN_	f	£	f	.f>
2030		2040			20	Ε Λ ·		_	000			
2030	*	2040		*	20	50 ·	*	· 2	060	•	*	
TCC TTA C	CGT TCC	ACA	ACT	ATG	ATG	ATT	CGA	CCT	 AT T	GAT	TTT	•
Ser Leu A	Arg Ser	Thr	Thr	Met	Met	Ile	Arg	Pro	Leu	Asp	Phe	: >
ff_	f_AN	G1 F	IBRI	NOGE	N-LI	KE D	OMAI	и	.f	f	f	_>

16/42 Figure 4A

	10		20		30		40	
*	* *	*	*	*	*	*	*	*
ATG TCT	GCA CTI	CTG	ATC CTA	GCT C	TT GTT	GGA GCT	GCA GTT Ala Val	Alas
met ser	a Ara rec	a TRV	PSTN ST	GNAL S	EQUENC	E a	aa	a>
aa		_a_ 1 \\ 1	IDIN DI	J	200			
50	•	60		70	1	80		90
*	*	*	*	.*	*	*	*	*
AGA GAC	TGT GC	GAA	GTA TTC	T AAA	CA GGA	CAC ACC	ACA AAT	GGC
Arg Asp	CAS VI	a Gin	TERTNO	Lys S Lys S	KE DOW	HIS THE ATN b	Thr Asn bb	b >
P		_MIG2	1 1DICINO	GEN E				
	100		. 110		120		130	•
*	*	*	*	•	*	*	*	*
ATC TAC	ACG TT.	A ACA	TTC CCT	AAT 1	CT ACA	GAA GAG	ATÇ AAG ı Ile Lys	GCC
ile iyr	Thr Le	DNG2	FIRRING	GEN-LI	LKE DOW	ATN b	bb_	b >
	J	_mvoz	PIDMINO	,GEM 12.				
140		150		160	0 '	170	•	180
•	*	*	. *	•	* *	*		*
TAC TGT	GAC AT	G GAA	GCT GGA	GGA (GC GGC	TGG AC	A ATT ATT	CAG
Tyr Cys	Asp Me	E GIU	ALA GIY	CEN-I.	LKE DOM STA GTA	TIP III.	r Ile Ile bb	p >
D	DD		Padramo	/OLIK 2				
	190		200		210		220	
*	*	*	·*		*	*	*	* .
CGA CGT	GAG GA	T GGC	AGC GT	r GAT	TTT CAC	AGG AC	T TGG AA	A GAA
Arg Arg	Giu As h h	ANG2	FIBRING	CASP OGEN-L	IKE DO	MAIN b	_bb	_b>
	~				•	-		
230		240)	25	0	260		270
*	*	*	*	a aam	*	* *	m mcc cm	C CCX
TAT AAA	GTG G	SA TTI	GGT AA	C CCL	TCA GG	A GAA TA	T TGG CT	u.Glv>
JAI DAS	b b	ANG2	FIBRIN	OGEN-I	IKE DO	MAIN_b_	_bb_	_b>
~							-	
.·	280	•	290		30	0	310	
	*	· ·	* *		,*	*	. *.	×
AAT GAG	TTT G	TT TCC	Cla Le	G ACT	AAT CA	n Gln Ai	GC TAT GT	l Leu>
Asn GI	b b	ANG:	2 FIBRIN	IOGEN-I	LIKE DO	MAIN_b_	_bb_	_b>
			•	•		•		
320		. 33	0 .	34	40	.35) .	360
*	*. • • • • • •	מא ממח	* አሮእር ጥር	י יר האא	CCC A	·π càc c	CT TAC TO	ግም ልግ
AAA AT	A CAC C	eu Lv	s Asp Tr	o Glu	Glv As	in Glu A	la Tyr Se	er Leu>
b_	_bb_	ANG	2 FIBRIN	OGEN-	LIKE DO	_d_nIAM	bb	b>
		•						
	370)	380)	3 9	90	400	•
*	3 C2m n	የጥር ጥጽ	יי רייירי ייי	- ጉል ልርጥ	CAA C	። ላል ሮጥሮ ል	AT TAT A	GG ATT
TAT GA ጥህታ ርገ	a car 1 u His I	he T	r Leu S	er Ser	Glu G	lu Leu A	sn Tyr A	rg Ile>
b_	_bb	ANG	2 FIBRI	NOGEN-	LIKE D	OMAIN_b_	bb_	b>

17/42 · Figure 4B

410)			420			43	30	_	4	140			450
CAC C	יירין רירין	AAA	* GGA	CTT.	ACA	eee	ACA	GCC	GGC	AAA	ATA	AGC	AGC	ATC
His Le	•				•								•	
		_	_			_			_	_)b		
													_	
,	k	4 (50 ★	*	4	170		*	480		*	49	• O	*
AGC C	AA (CCA	GGA	AAT	GAT	TTT	AGC	ACA	AAG	GAT	GĠA	GAC	AAC	GAC
Ser G	ln i	Pro	Gly	Asn	Asp	Phe	Ser	Thr	Lys	Asp	Gly	Asp	Asn	Asp>
b_	—p.	,	D	ANG2	FIB	RINO	GEN-I	LIKE	DOM	AIN_	b1	t	,;	>>
50	0			510			5:	20			530			540
,	*		*	*		*		*	*		*		*	*
AAA T														
b_														
										·				
	*	5.	50 *	*	!	560		*	570		*	. 58	30 *	*
TTT G	AΤ	GCA	TGT	GGT	ССТ	TCC	AAC	TTG	AAC	GGA	ATG	TAC.	TAT	CCA
Phe A	sp	Ala	Cys	Gly	Pro	Ser	Asn	Leu	Asn	Gly	Met	Tyr	Tyr	Pro>
b_	b		b	ANG2	FIB	RINO	GEN-	LIKE	DOM	AIN_	b	b!	اا	b>
. 59	0.			600			6	10			620			630
	*		*	• •		*		*	*		*		*	*
CAG A														
Gln A														p>
						y-					•			 .
		- 6	40		•	650		•	660		*	6	70	
TGG A	AA	GGC	TCA	GGC	TAT	TCC	CTC	AAG	GCC	ACA	ACC	ATG	ATG	ATC
Trp L	ys	Gly	Ser	Gly	Tyr	Ser	Lev	Lys	: Ala	Thr	Thr	Met	Met	Ile>
p_	—_t	·	p	_ANG2	FIE	RINC	GEN-	LIKE	E DOM	IAIN_	ъ	.b	р	.b>
68	0			690)		7	00			710			720
	*		*	*	,	, ★		*	•	٠	*		*	*
CGA C						GGC	ccc	GGC	GAG	CC(C AAZ	L TCI	TGT	GAC
Arg I			_) Pre							•			
					_	/ Gly	Pro	Gly	/>					
						_GGP(BR:	<u> </u>	_>_	. Dr	O Tar	- 60-	. 0.,,	Asp>
									GI	_d				_d>
•										_		_		
	*	•	730 *	,	4	.740		*	75	0 ★	*	7	760 *	*
AAA	ACT	CA	C AC	A TG	c cc	A CC	G TG	c ˈcc	A GC	A CC	T GA	A CTO	CTO	GGG
														ı Gly>
d		d	_d	_d	_a	_d	FC T	AG	_a	_a	_a	_a	_a	_d>
. 7	70			78	0			790		٠	800			810
	*		*	_	*	*	_ =-		_	*	*		*	*
														C CTC r Leu>
														_d>

18/42 Figure 4C

		Ľ	TRUL	* 4 6							
1	820		830			840			85	0	
*	*	*	*		*	*		*		*	*
ATG ATC TCC	CGG AC	יר רריי	CAG	CTC	A C A	ጥርር	СТС	CTC	GTG /	באר	CTC
Met Ile Ser	Ara Th	or Dro	Glu	Unl C	mb-	Cure	nej Gro	1701	Ual V) and	Unla
7 A	4 4	FIO	4 EC	AGT	7 11T	Cys	vaı	va.	vaı .	rsp	vai>
dd	·uu_		urC	TAG	С	0		a	a	c	·>
0.60					_		_				
860	8.	70		88	0		8	90			900
*	*	*	•		*	*	• .	*.		*	*
AGC CAC GAA											
Ser His Glu	Asp P	ro Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val .	qzA	Gly>
aa	.dd_	d	dFC	TAG	ċ	1d	ـــــــــــــــــــــــــــــــــــــ	<u></u> d	La	c	i>
•											
و.	10		920			930			94	0	
. *	*	* .	*		*	*		*		*	*
GTG GAG GTG	CAT A	AT GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC
Val Glu Val	His A	sn Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu:	Ġln	Tyr>
aa	d d	đ	d FC	TAG		i d	i c	a d	a d		i >
	·										
950	9	60		97	0		. c	980			990
*	*	*	*		*	*		*		*	*
AAC AGC ACC	י אר כי	ርጥ ርጥር	CTY	ACC	CITY	CTC	NCC.	CITIC	CITYC:	CAC	CAC
Asn Ser Thr											
dd											
		a	α	. IAG	<u>ښ</u>	مـــــــد	٠	J	10		<u>></u>
1,	000		010								
, I(. 4		010			1020			103	U	
*		*	*		*	. *		*		*	*
GAC TGG CTC	AAT G	GC AAG	GAG	TAC	AAG	TGC	AAG	GTC	TCC	AAC	AAA
Asp Trp Leu											
dd	_dd_	đ	dF	TAG	:	d0	٠	d0	dc	<u> </u>	d>
						·.	-	٠.	17		
1040	10	50		106	0		1	070		•	1080
*	*	*	*		*	*		*		*	*
GCC CTC CC											
Ala Leu Pro											
dd	_dd_	d	_dF	CAT.	3	d	a	a _	đ¢	<u>.</u> ــــــــــــــــــــــــــــــــــــ	d>
	1						٠.			:	٠
- 10	090	1	100			1110		-	112	20	,
*	* •	*	*		·* ·	*		**		* .	. *
CAG CCC CG	A GAA C	CA CAC	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT
Gln Pro Ar											
dd	d d	ď	d F	ር ጥል(3	ď	ď	d .	a	 1	d >
								. ~			
1130	. 11	L 4 0	•	11	50			160			1170
*	*	*	*		*	*	. 🚣	*		*	*
GAG CTG AC	CAAG	אכ כאַנ	2 CTC	AGC	CTC	ACC	TCC	CTC	CTC	תתת	CCC
Glu Leu Th											
dd			_u	CIA	<u> </u>	.u	.u	٠	·u	u	-u>
·	180		1100			1200					
	*		1190			1200			12	T O	_
mmo m	 	~				·		, . . -		~	*
TTC TAT CC											
Phe Tyr Pr											
dd	_ad.	a	_aF	TA	G	_a	'q	_d	_q	d	_d>
1220	1	230		12	40		1	L250			1260
•	*	* .	*		*	•		*		. *	*
CCG GAG AA											
Pro Glu As											
dd	aa	a	_at	CIA	·G					·u	_a?

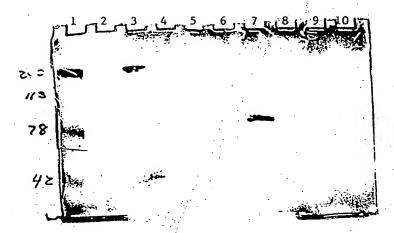
19/42 Figure 4D

	12	70		12	В0		. 1	.290		_	130)	
GGC TC	- mmc	*	. * .cmc :	maic :	* **	N N C	~ CTC	אַרַר ק	CTC	CAC	DA4	AGC	AGG
Gly Se	. TTC	Dhe	Len	TUY	Ser 1	nno Ivs	Len	Thr	Val	Aso	Lvs :	Ser	Arg>
dry ser	_d	d d	a d	d.	FC	TAG	c	i c	1d	d	a		- E
		·											
1310			L320			133	0		13	40		1	1350
*		*	*		*		*	. *	:	* .		*	*
TGG CA	G CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT
Trp Gl	n Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	.Val	Met	His	Glu	Ala>
d	_d	d	dd	d	FC	TAC	°	dC	,—,c	ıc	ıa	(a >
	12	C 0		12	70			1380			139	n	
*	13	*	*	. 13	/ U .★.		*	*		*		*	*
CTG CA	C ÄAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT
Leu Hi	s Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly>
a_	_d	d	a6	id	FC	TAC	3	a	dd	٠٥	ia	نسنا	d>
	•												
1400	1	•	1410	**		14:	20		14	130			1440
*		*	. *		*		* .	*	~ ~	. *.	com	*	~~~
AAA GG	C GG1	GGC	GGT	TCT	GGC	GCG	CCT	AGA	GAC	TGT	GCT	GAA	GTA
Lys>													
	y Gly	, G1v	Glv	Ser	Glv	Ala	Pro	· >					
	e							>					
							· :	Arg	Asp	Cys	Ala	Glu	val>
	•								ANG2	FIB	RINO	3EN−	>
			200			.*	· · · .		٠.				•
-	. 14	150		. 14	460			1470)		14	30	
TTC A	r Namori	. * 		ACC	ת את	א א מ	י ממר	י איזיא מיי	יישרי	ACG	מידים	ACI	א שיירי
Phe Ly	AA ICA	r Gli	. CAC	Thr	Thr	Asn	Glv	Tle	Tvr	Thr	Leu	Thi	c Phe>
f f	f f	f	ANG2	FIB	RINO	GEN-	LIKE	DON 3	IAIN_	£	£	£	_f>
			-						•			•	
149	0		1500			15	10		1	520		:	1530
	*	*	*		*		*	1	k.	*		*	. *
CCT A	AT TC	T AC	A GAA	GAG	ATC	AAG	GCC	AT.	TGI	GAC	ATG	GA	A GCT
Pro A	sn Se	r Th	r Glu	Glu	Ile	Lys	T TV	e noi	с Суз их тат	E ASE	, mec	e GTI	u Ala>
t-	—±—	_ <u>+</u>	_ANG2	FIB	KINO	GEN-	-LIK	E DOI	MAIN_	. ¹			_£>
•	1	540		1	550			156	0		15	70	•
	*	*	*	•	*		*		*	*		*	*
GGA G	GA GG	C GG	G TG	ACA	TTA .	'AT	r CA	G CG	A CG	GAO	G GAT	, GG	C AGC
Gly G	ly Gl	y G1	y Trp	Thr	Ile	:Ile	e Gl :	n Ar	g Arg	g Glı	ı Asp	G1	y Ser>
f_	f	f	_ANG2	FIE	BRINC	GEN-	-LIK	E DO	MAIN_	_£	_£	.£	_ f >
	_					-				1610			1620
158	10		1590) •		1	600		*	1610		*	1620
ല വ	። የአጥ ጥባ	יעה כי יי	ה אפני	" ጌ ልሮባ	יי יי ייינור	44 S	A GA	а та	т аа	A GT	G GGZ	х тт	T GGT
Val A	sp Pl	ne Gl	n Arc	Th:	Tri) Lv	s Gl	u Ty	r Ly	s Va	1 Gly	Ph	e Gly:
£	f :	£	ANG	2 FII	BRING	OGEN	-LIK	E DO	MAIN	_f	_£	_£	f:
	:	1630			1640			165	0		1	660	
	*	.*		*	*	:	*		*	* ~		*	*
AAC (CT T	CA GO	SA GA	A TA	T TG	G CT	G GG	A A	T GA	G TT	T GT	TC	CG CAA
													er Gln
f_	f	£	_ANG:	2 FIE	BRING	OGEN	-LIK	E DO	MAIN	_f	_f	_f	_£ :

20/42 Figure 4E

	Berc 45		
1670 1680	1690	1700	1710
CMC 30m 33m 030 m3	* *	* *	• •
CTG ACT AAT CAG CAA	CGC TAT GTG CT	T AAA ATA CAC	CTT AAA GAC
Leu Thr Asn Gln Glr	Arg Tyr Val Le	u Lys Ile His	Leu Lys Asp>
ffANG2	FIBRINOGEN-LIK	E DOMAIN_f	tf>
1720	1730	1740	1750
* * *	* *	* *	* *
TGG GAA GGG AAT GAG	GCT TAC TCA TT	G TAT GAA CAT	TTC TAT CTC
Trp Glu Gly Asn Glu	Ala Tyr Ser Le	u Tyr Glu His	Phe Tyr Leus
fffANG2	FIBRINOGEN-LIK	E DOMAIN_f	ff_ f >
1760 1770	1780	1790	1800
* * *	* *	* *	* *
TCA AGT GAA GAA CTC	AAT TAT AGG AT	T CAC CTT AAA	GGA CTT ACA
Ser Ser Glu Glu Leu	Asn Tyr Arg Il	e His Leu Lys	Gly Leu Thr>
ffANG2	FIBRINOGEN-LIK	E DOMAIN_f	ff>
1810	1820	1020	
* . *	1020	1830	1840
GGG ACA GCC GGC AAZ	. איים אכר אכר איי	ר אכיכי כאא פיכא	CC1 33m c1m
Gly Thr Ala Gly Lys	Ile Ser Ser Il	e Ser Cln Pro	GGA AAT GAT
ffANG2	FIBRINGEN-LIK	E DOMATN F '	GIY ASH ASP>
		DOININ_I	·
1850 1860	1870	1880	1890
* *		* *	* *
TTT AGC ACA AAG GAT	GGA GAC AAC GA	C AAA TGT ATT	TGC AAA TGT
Phe Ser Thr Lys Asp	Gly Asp Asn As	D Lys Cys Ile	CVS LVS CVS>
ffANG2	FIBRINOGEN-LIK	E DOMAIN_f	ff>
		*	
1900	1910	1920	1930
TO CAN ATC CON ACT	CO) COO MOO MO	*	* *
TCA CAA ATG CTA ACA	GGA GGC TGG TG	G TIT GAT GCA	TGT GGT CCT
Ser Gln Met Leu Thr	CIY GIY TID TI	b bue wash wire	Cys Gly Pro>
	FIBRINOGEN-LIK	E DUMAIN_I	rr>
1940 1950	1960	1970	1980
**	*	* *	* *
TCC AAC TTG AAC GG	ATG TAC TAT CO	A CAG AGG CAG	AAC ACA AAT
Ser Asn Leu Asn Gly	Met Tyr Tyr Pr	o Gln Arg Gln	Asn Thr Asn>
fffANG	FIBRINOGEN-LIK	E DOMAIN_f	ff
1990	2000	2010	2020
110 mmc 110 ccc 1		* *	*
AAG TTC AAC GGC ATT	AAA TGG TAC TA	C TGG AAA GGC	TCA GGC TAT
Lys Phe Asn Gly Ile	Lys Trp Tyr Ty	r Trp Lys Gly	Ser Gly Tyr>
ffANG	: LIBKINOGEW-TIK	E DOMAIN_f	ff>
2030 2040	2050	2060	2020
* *	* * *	* *	2070
TCG CTC AAG GCC AC	ACC ATG ATG AT	C CGA CCA GCA	GAT TTC TCA
Ser Leu Lys Ala Th	Thr Met Met Il	e Arg Pro Ala	Asp Phe>
ff_ANG2 1	FIBRINOGEN-LIKE	DOMAINf_	ff>

Figure 5



Angl-FD-Fc-FD

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Figure 6 Molar Mass vs. Volume

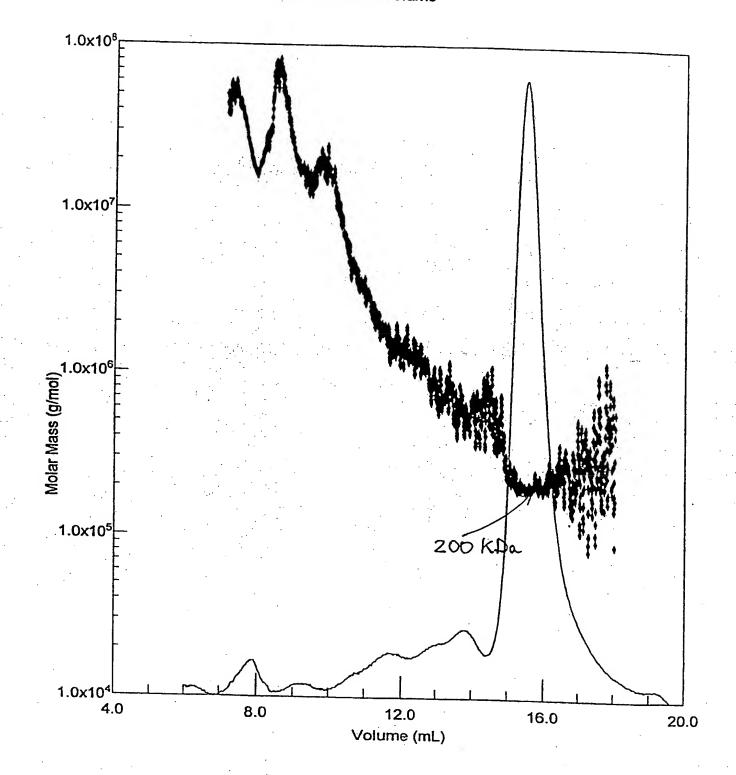
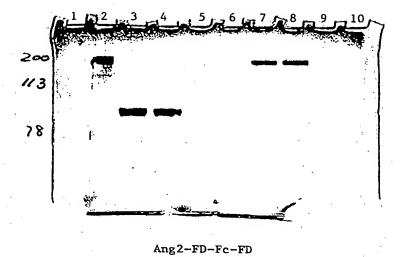
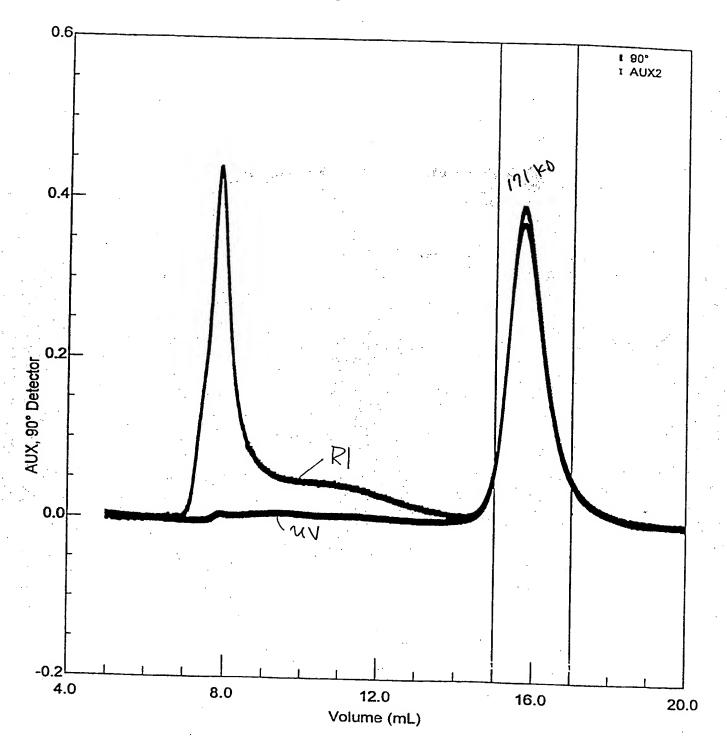


Figure 7



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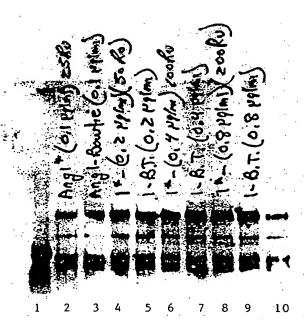
Figure 8



SVSDOCID: SIND : MOZENSA:

WO 00/37642

Figure 9



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Figure 10

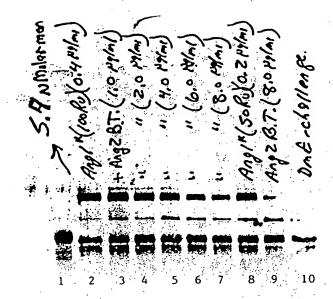


Figure 11

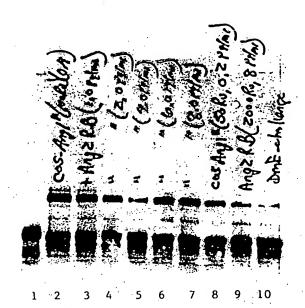


Figure 12

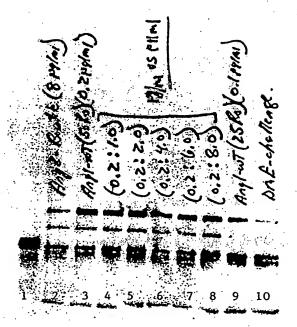


Figure 13

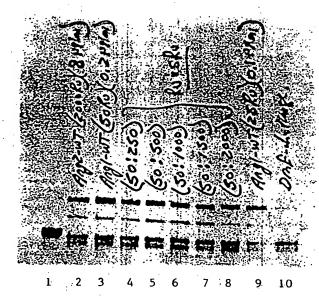


Figure 14A

	10	20		30	4	0
ATG GCT CG	* * * OGG	CAG CGT T	* 'GG CTC -	* GGC AAG	* ፕሮር ርጥጥ	* *
Met Ala Ar						
aa_E	LK-L ECTO	OMAIN 1 (WITH SI	GNAL PE	PTIDE)a	a>
50	60		70		80	90
*	* *	* .	*	*	*	* *
ATG GTC GT						
Met Val Va a a E	ELK-L ECTO					
•	1,00	110		120	13	30
AAC CTG GA	AG CCC GTA	TCC TGG A	AGC TCC	CTC AAC	CCC AAG	TTC CTG
Asn Leu G	•					
aa_I	ELK-L ECTO	DOMAIN 1	(WITH SI	GNAL PE	PTIDE)a	aa>
140	150		160	: .	170	180
*	u 💌 Šugt		*	. •	*	* *
	AG GGC TTG					
	ys Gly Leu ELK-L ECTO					
			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	7.7		~·
	190	200	. 1	210	. 2:	20
GAC ATC A	IC TGC CCC	CGA GCA	GAA GCA	GGG CGG	CCC TAT	GAG TAC
A	le Cys Pro					
aa	ELK-L ECTO	DOMAIN 1	(WITH S	IGNAL PE	PTIDE)	aa>
230	240		250		260	270
*	*	*	*	*		* *
	TG TAC CTG					
	eu Tyr Leu ELK-L ECTO					
			•			····
•	280	290		300	3	10
ACA GTT C	TC GAC CCC	AAC GTG	TTG GTC	ACC TG	AAT AGG	CCA GAG
	eu Asp Pro					
aa_	ELK-L ECTO	DOMAIN 1	(WITH S	IGNAL PI	EPTIDE)	.aa>
320	- 330		340		350	360
•	• •	*	*	*	•	* *
	TA CGC TTT le Arg Phe					
	ELK-L ECTO					
•	370	380	*	390 *	* *	100
TAC ATG C	GC CTG GAG	TTC AAG	AAG CAC	CAT GA	T TAC TAC	ATT ACC
Tyr Met G	Sly Leu Glu	Phe Lys	Lys His	His As	p Tyr Ty	Ile Thr>
au_	ELK-L ECTO	DOMAIN 1	(WITH S	IGNAL P	EPTIDE)_	_a;

Figure 14B

410	420	4	30	440	450
TCA ACA TCC	AAT GGA AGO	CTG GAG	GGG CTG	GAA AAC	CGG GAG GGC
Ser Thr Ser					
)a>
4.6	50	470	480		490
*	* *	*	* *	•	* *
GGT GTG TGC					
			_		Lys Val Gly>)aa
			010		,
500	510	5	20	530	540
CAA GAT CCC	AAT GCT GTG	ACG CCT	GAG CAG	CTG ACT	ACC AGC AGG
					Thr Ser Arg>
a_a_ELI	K-L ECTODOMA	IN i (WI	TH SIGNA	L PEPTIDE)a>
55	50	560	570		580
*	* *	*	* *	*	•
CCC AGC AAG					
	K-L ECTODOMA				Thr Gln Ala>
			Dadiii		/
590	600	ε	10	620	630
CCT GGT AGT	CGG GGC TCG	י אי . רידופ פפיז	י האר יתריו וי האר יתריו	የ ርልጥ ርርር	AAC CAT CAC
					Lys His Glu>
					:)aa>
6	40	650	660)	670
* =	* = *	*	*	• .	
	CAG GAA GAG				
					Ser Gly Gly>
680	690		700	710	720
AGC AGC GGG	GAC CCT GA	r GGC TT	TTC AAG	TCC AAG	GGC CCG GGT
Ser Ser Gly	Asp Pro As	p Gly Ph	e Phe Asi	Ser Lys:	•
ELK-L EC	TODOMAIN 1	(WITH SI	GNAL PEP	ride):	
			•		Gly Pro Gly>
	-20				
*	* *	740 *	75.º ★	D ★ *	760
AAG AAC CTG	GAG CCC GT	A TCC TG	G AGC TC	CTC AAC	CCC AAG TTC
Lys Asn Leu	Glu Pro Va	l Ser Tr	p Ser Se	r Leu Asn	Pro Lys Phe>
cc	CEDK-D EC	TODOMATN	2 (NO S	IGNAL)	cc>
770	780		790	. 800	810
* CTC &CT CCC	* * *	· *	* C 30800 CC	* * *	* *
					GGA GAC AAG Gly Asp Lys>
					cc>
				,	

32/42 Figure 14C

820	830	840	850
* * *	*	* *	* * *
CTG GAC ATC ATC TGC			
Leu Asp Ile Ile Cys	Pro Arg Ala	Glu Ala Gly	Arg Pro Tyr Glu>
ccELK-L	ECTODOMAIN	2 (NO SIGNAL)	cc>
250			
860 870		80 1	390 900
TAC TAC AAG CTG TAC	רתה מתה רהה	רכיד מאכ כאכ	* * * *
Tyr Tyr Lys Leu Tyr			
ccc_ELK-L			
· ·		•	
910	920	930	940
* * * *	*	* *	* * *
AGC ACA GTT CTC GAC Ser Thr Val Leu Asp	CCC AAC GTG	TTG GTC ACC	TGC AAT AGG CCA
ccELK-L	ECTODOMAIN	2 (NO STONAL	Cys Asn Arg Pro>
		- (NO SIGNAL	
950 960	9	70	980 990
* * *	*	* *	* * *
GAG CAG GAA ATA CGC			
Glu Gln Glu Ile Arg			
ccELK-L	ECTODOMAIN	2 (NO SIGNAL)cc>
1000	1010	1020	1030
* *	. *	*	* * *
AAC TAC ATG GGC CTG	GAG TTC AAG	AAG CAC CAT	GAT TAC TAC ATT
Asn Tyr Met Gly Leu	Glu Phe Lys	Lys His His	Asp Tyr Tyr Ile>
cccELK-L	ECTODOMAIN	2 (NO SIGNAL)cc>
1040 1050	10	60 1	070 1080
* *	*	* *	* *
ACC TCA ACA TCC AAT	GGA AGC CTG	GAG GGG CTG	GAA AAC CGG GAG
Thr Ser Thr Ser Asn	Gly Ser Lev	Glu Gly Leu	Glu Asn Arg Glu>
cccELK-L	ECTODOMAIN	2 (NO SIGNAL)cc>
1090	1100	1110	
	1100		1120
* * *	*	* *	1120
GGC GGT GTG TGC CGC	ACA CGC ACC	ATG AAG ATC	* * * * ATC ATG AAG GTT
Gly Gly Val Cys Arg	ACA CGC ACC	* * * * C ATG AAG ATC Met Lys Ile	ATC ATG AAG GTT Ile Met Lys Val>
	ACA CGC ACC	* * * * C ATG AAG ATC Met Lys Ile	ATC ATG AAG GTT Ile Met Lys Val>
Gly Gly Val Cys Arg	ACA CGC ACC Thr Arg Thi ECTODOMAIN	ATG AAG ATC Met Lys Ile 2 (NO SIGNAL	ATC ATG AAG GTT Ile Met Lys Val>
Gly Gly Val Cys Arg	ACA CGC ACC Thr Arg Thi ECTODOMAIN	ATG AAG ATC Met Lys Ile 2 (NO SIGNAL	ATC ATG AAG GTT Ile Met Lys Val>
Gly Gly Val Cys Argccc_ELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN	ATG AAG ATC Met Lys lle 2 (NO SIGNAL L50 1	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys ArgcccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr	ATG AAG ATC MET LYS Ile (NO SIGNAL L50 1 CCT GAG CAG	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys Argccc_ELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr	ATG AAG ATC MET LYS Ile (NO SIGNAL L50 1 CCT GAG CAG	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys ArgcccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr ECTODOMAIN	ATG AAG ATC MET LYS Ile (NO SIGNAL L50 1 CCT GAG CAG Pro Glu Gln 2 (NO SIGNAL	ATC ATG AAG GTT Ile Met Lys Val>)cc> 160
Gly Gly Val Cys ArgcccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr	ATG AAG ATC MET LYS Ile (NO SIGNAL L50 1 CCT GAG CAG	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys ArgcccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC	C ATG AAG ATC MET LYS Ile 2 (NO SIGNAL L50 1 C CCT GAG CAG Pro Glu Gln 2 (NO SIGNAL L200 C ACT GTC AAG	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys Arg cccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC Ala Asp Asi	C ATG AAG ATC MET LYS Ile 2 (NO SIGNAL L50 1 C CCT GAG CAG Pro Glu Gln 2 (NO SIGNAL L200 C ACT GTC AAG Thr Val Lys	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys ArgcccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC Ala Asp Asi	C ATG AAG ATC MET LYS Ile 2 (NO SIGNAL L50 1 C CCT GAG CAG Pro Glu Gln 2 (NO SIGNAL L200 C ACT GTC AAG Thr Val Lys	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys Arg cccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC Ala Asp Ass	ATG AAG ATC MET LYS Ile (NO SIGNAL LSO 1 COT GAG CAG Pro Glu Gln (NO SIGNAL L200 COT ACT GTC AAG Thr Val Lys (NO SIGNAL	ATC ATG AAG GTT Ile Met Lys Val>)ccc> 160
Gly Gly Val Cys Arg cccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN 11 GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC Ala Asp Ass	ATG AAG ATC MET LYS Ile (NO SIGNAL LSO 1 COT GAG CAG Pro Glu Gln (NO SIGNAL L200 COT ACT GTC AAG Thr Val Lys (NO SIGNAL	ATC ATG AAG GTT Ile Met Lys Val> cc> 160
Gly Gly Val Cys Arg cccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC Ala Asp Ass ECTODOMAIN 1: GCT GTG ACC ALC ALC ALC ALC ALC ALC ALC ALC ALC	C ATG AAG ATC MET LYS Ile 2 (NO SIGNAL L50 1 G CCT GAG CAG Pro Glu Gln 2 (NO SIGNAL L200 C ACT GTC AAG Thr Val Lys 2 (NO SIGNAL L240 1 G GGT GAC TCT	ATC ATG AAG GTT Ile Met Lys Val>)ccc> 160
Gly Gly Val Cys Arg cccELK-L 1130	ACA CGC ACC Thr Arg Thr ECTODOMAIN GCT GTG ACC Ala Val Thr ECTODOMAIN 1190 GCA GAC AAC Ala Asp Asr ECTODOMAIN 1: GGC GCC CTC GGly Ser Lee	C ATG AAG ATC MET LYS Ile 2 (NO SIGNAL L50 1 G CCT GAG CAG Pro Glu Gln 2 (NO SIGNAL L200 C ACT GTC AAG Thr Val Lys 2 (NO SIGNAL L240 1 G GGT GAC TCT U Gly Asp Ser	ATC ATG AAG GTT Ile Met Lys Val>)ccc> 160

33/42 Figure 14D

1270	128	0	1290	1300	
* *	• .	* *	18	* *	*
GAG ACT GTG A	AC CAG GAA G	AG AAG AGT	GGC CCA G	GT GCA AGT	GGG
Glu Thr Val As					
ccc_					
1310	1320	1330	134	.0	1350
* *	. *	* *	*	* *	*
GGC AGC AGC G					CCG
Gly Ser Ser G					
c_·_cELK	-L ECTODOMA	[N 2 (NO S]	GNAL)c_		
	,	**			Pro>
					_d>
1260	12.	7.0	1380	1390	
1360	13	/U * *	1290	* *	*
GGC GAG CCC A	ልል ጥርጥ ጥርጥ (ממ ממב	ר באב אבא יו	ייני ככא ככנ	TCC
Gly>	AN ICI IGI (inc nun ne	che hen	ide cen eet	
>					
	ys Ser Cys	Asp Lys Th	r His Thr (Cys Pro Pro	Cys>
	eeHU				
			(· .	
1400	1410	1420	.14	30	1440
* * *	* .	*.	*	. *	*
CCA GCA CCT G					
Pro Ala Pro G					
eee	eHUMA	N IGG1 FC	TAGee	ee	_e>
		CO	1.470	1400	•
1450) 14	• •	1470	1480	*
CCA AAA CCC	את כאת אתר	כייר אייר איי	יר זירר רפפ	ACC CCT GA	G. GTC
Pro Lys Pro I	ws Asp Thr	Leu Met Il	e Ser Arg	Thr Pro Gl	u Val>
eee	e HUMA	N IGG1 FC	TAG e e	ee_	_e>
1490	1500	1510	15	20 .	1530
*		★ 5.2 *	*	* *	*
ACA TGC GTG					
Thr Cys Val	Val Val Asp	Val Ser Hi	ls Glu Asp	Pro Glu Va	l Lys>
ee	eHUM <i>I</i>	M IGG1 FC	TAGee	ee_	_e>
154	0 19	550	1560	1570	•
TTC AAC TGG	m.a ama a.a	occ omc c	,	NAM CCC N	
Phe Asn Trp	TAC GIG GAC	Cly Val C	lu Val Hic	An Ala I.i	e Thro
	e_HUM				
		1001 10			
1580	1590	1600	. 10	610	1620
. •	• .•	* *	•	* *	*
AAG CCG CGG					
Lys Pro Arg					
eee	ее НՄМ	AN IGG1 FC	TAGe	ee_	e:
163	30 1	640	1650	1660	
		* *	*	* *	*
GTC CTC ACC					
Val Leu Thr	val Leu His				

34/42 Figure 14E

1670	1680		1690	_	1700	1710
AAG TGC AAG	GTC TCC	AAC AAA	GCC CTC	CCA GC	C CCC AT	C GAG AAA
Lys Cys Lys						
ee	_ee	_HUMAN I	GG1 FC T	AGe_	_ee	_e>
17	720	1730		1740	1	750
. *	* *	*	*	*	*	* *
ACC ATC TCC						•
ee						
1760	1770		1780		1790	1800
*	* *	*	*	*	*	* *
ACC CTG CC						
Thr Leu Pro						
ee_	_ee	_HOMAN I	GGI FC 1	.AGe	_ее_	_ee>
·. 1	810	1820		1830	. 1	840
*	* · *	*	* .	*	*	* *
CTG ACC TG						
						_ee>
1050	. 1000		1070	•	1000	. 1800
1850 *	1860	*	1870 *	*	1880	1890
GAG TGG GA						
						ys Thr Thr>
ee	_ee	NOMAN .	IGGI FC	LAGE_	ee	ee>
1	900	1910		1920		1930
CCT CCC GT	ים רייים מאני אים רייים מאני	. * . TOO GA		י ארארוי ידי איז יארארוי י	אר כיוזיכי יוזי א	* * * * * * * * * * * * * * * * * * * *
./						yr Ser Lys>
						ee>
1940	1950		1960		1970	1980
± ±	* *	· .	+	*	*	* *
						TC TTC TCA
						al Phe Ser> ee>
			1001 10			
1	L990	2000		2010		2020
TGC TCC G	rg atg ca	T GAG GC	T CTG CA	C AAC C	AC TAC A	CG CAG AAG
Cys Ser Va	al Met Hi	s Glu Al	a Leu Hi	s Asn H	is Tyr T	hr Gln Lys>
ee_	ee	HUMAN	IGG1 FC	TAGe_	ee_	e>
2030	204	0	2050			
*	*	* *	*			
AGC CTC TO					•	
eH						
•			_			

35/42 ..Figure 15A

10	20	30	4	0
ATG GCC ATG GCC Met Ala Met Ala a EPHRIN-B2	Arg Ser Arg		l Trp Lys	Tyr Cys>
50	60	70	80	90
* *	* *	*	*	* *
TGG GGA CTT TTG				
Trp Gly Leu Leua_EPHRIN-B2				
ab: iix1x-b2	ECTO DOMAIN	I I (WIIN SIGNA	L FEFTIDE,	a
100	. 110	120	13	0 .
ATA GTT TTA GAG	* * * ' CCT ATC TAC	# # ##################################	*	* *
Ile Val Leu Glu		and the second s		
a_EPHRIN-B2				
140	150			
140	150	160	170	180
CTA CCC GGA CA	GGC CTG GTA	CTA TAC CCA CA	G ATA GGA	GAC AAA
Leu Pro Gly Gl				
a_EPHRIN-B	ECTO DOMAII	1 1 (WITH SIGNA	AL PEPTIDE)	a>
190	200	210	22	.n
* *	* *	* *	*	* *
TTG GAT ATT AT				
Leu Asp Ile Ile				
a_EPHKIN-B	z ECTO DOMATI	N 1 (WITH SIGN	AL PEPTIDE)	a>
230	240	250	260	270
*	* *	* *	*	*
TAT GAA TAT TA Tyr Glu Tyr Ty				
a_EPHRIN-B				
280	290	300	31	10
AGA TGC ACA AT	T AAG AAG GAG	אד ארר ררה ר	דה כדר AAC	TOT GCC
Arg Cys Thr Il				
		N 1 (WITH SIGN		
220	220	340		260
320	330 .	340	350 *	360
AGA CCA GAC CA	A GAT GTG AAA	TTC ACC ATC A	AG TTT CAA	GAA TTC
Arg Pro Asp Gl	7		-	
a_EPHRIN-B	2 ECTO DOMAI	N 1 (WITH SIGN	AL PEPTIDE)a>
3 <u>7</u> 0	380	390	.4	00
	*		*	•
AGC CCT AAC CT				
		Glu Phe Gln L N l (WITH SIGN	-	
		,		

36/42 Figure 15B

	410	•	420		430		440		450
	TAC ATT	ата тст	ACA TC	A AAT	GGG TCT	TTG GAG	GGC C	TG GAT	AAC
	Tyr Ile								
	aEP								
		460		470		400	•	490	
	*	460	*	470 *	*	480 *	*	4.50	*
	CAG GAG								
	Gln Glu	Gly Gly	Val Cy	s Gln	Thr Arg	Ala Met	Lys I	le Leu	Met>
	aEP	HRIN-B2	ECTO	DOMAIN	T (MITH	SIGNAL	, bebil	LDE)a	>
	500		510		520		530		540
	*	*	* .	* .	*	*	*	*	*
	AAA GTT Lys Val								
		HRIN-B2							
				5.50		570		500	
	*	550 *	**	560 *	*	570 *	*	580	*
	GGT CCA								
	Gly Pro								
	aEI	PHRIN-B2	ECTO	DOMAIN	1 1 (WIT)	H SIGNAL	- PEPT	IDE)	a>
	- 590		600		610	:	620		630
	*	*	•	*	* *	*	*	*	*
:		ACA ACA							
		PHRIN-B2			_				
	•	640 *	*	650 *	*	660 *	*	670 *	*
		GGC AAC							
		Gly Asn							
	aE	PHRIN-B2	ЕСТО	DOMAII	N I (MIT	H SIGNA	L PEP1	.IDE)	a>
	680		690		700		710		720
	*	* .	*	*	*	* :	*	*	*.
	GGC CCG	GGA ATA	A GTT T	ra Gag	CCT ATC	TAC TG	G AAT	TCC TCG	AAC
	b			•		•			•
					Pro Ile	_	_		
		٠	_EPHRIN	-B2 E	CTO DOMA	AIN 2 (WITHOU	JE SIGNA	·>
		730		740		750		.760	
	maa	*	*		*	*	*	*	*
		TTT CT							
		RIN-B2			2 (WITH				
	220		700		700		800		010
	770		780		790 •		*		810
		TT AAA :							
		Lys Le							
	EP	RIN-BS	ECTO C	NIANCE	Z (WIT)	HOUT SI	MAL P	EPTIDE),	c>

37/42 Figure 15C

830 850 GTT GGC CAG TAT GAA TAT TAT AAA GTT TAT ATG GTT GAT AAA GAC Val Gly Gln Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp> _EPHRIN-B2 ECTO_DOMAIN 2 (WITHOUT SIGNAL PEPTIDE)_e___> 860 870 880 900 CAA GCA GAC AGA TGC ACA ATT AAG AAG GAG AAT ACC CCG CTG CTC Gln Ala Asp Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu> ___EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE) _e ___> . 920 930 AAC TGT GCC AGA CCA GAC CAA GAT GTG AAA TTC ACC ATC AAG TTT Asn Cys Ala Arg Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe> __EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE)_e__> . 970 960 CAA GAA TTC AGC CCT AAC CTC TGG GGT CTA GAA TTT CAG AAG AAC Gln Glu Phe Ser Pro Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn> _EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE)_e___> 1010 1020 AAA GAT TAC TAC ATT ATA TCT ACA TCA AAT GGG TCT TTG GAG GGC Lys Asp Tyr Tyr Ile Ile Ser Thr Ser Asn Gly Ser Leu Glu Gly> ___EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE) _e___> 1050 1060 1070 CTG GAT AAC CAG GAG GGA GGG GTG TGC CAG ACA AGA GCC ATG AAG Leu Asp Asn Gln Glu Gly Gly Val Cys Gln Thr Arg Ala Met Lys> ____EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE)_e___> 1100 -1110 1120 . ATC CTC ATG AAA GTT GGA CAA GAT GCA AGT TCT GCT GGA TCA GCC Ile Leu Met Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser Ala> ___EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE) _e___> 1130 AGG AAT CAC GGT CCA ACA AGA CGC CCA GAG CTA GAA GCT GGT ACA Arg Asn His Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr> ____EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE) _e___> 1190 1200 AAT GGG AGA AGT TCA ACA ACA AGT CCC TTT GTG AAG CCA AAT CCA Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro> ____EPHRIN-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE) _e__ 1230 GGT TCT AGC ACC GAT GGC AAC AGC GCG GGG CAT TCC GGG AAC AAT Gly Ser Ser Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn> EPHRIH-B2 ECTO DOMAIN 2 (WITHOUT SIGNAL PEPTIDE) 6 ...

38/42 Figure 15D

1270 1280 1290 1300 CTC CTG GGG G GC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC Glu Pro Lys Ser Cys Asp Lys Thr His> _c___HUMAN IGG1 FC TAG__c__c__> Gly Pro Gly> _d__d__d__> Leu Leu Gly Xxx> ___e__e_> 1310 ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser> 1370 GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser> _c__c__HUMAN IGG1 FC TAG__c__c__c_ 1410 1420 1430 CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu> c__c_ HUMAN IGG1 FC TAG_c_c_c_c_c_ GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val> _c__c__HUMAN IGG1 FC TAG__c__c__c_ 1510 1490 1500 1520 CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr> _c__c__c__HUMAN IGG1 FC TAG__c__c__c__c___> 1560 TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu> ___c__c__c__HUMAN IGG1 FC TAG__c__c__c__c__> 1580 1590 1600 1610 AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro> _c__c__c__c__HUMAN IGG1 FC TAG__c__c__c__c___ 1630 1640 1650 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg-LC. C. C. HUMAN IGG1 FC TAG. C. C. C. C. C.

39/42 Figure 15E

1	670			1680	1		16	90		1	700			1710
	*		*	*		*		*	*				*	
GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	CAT	CNC	CmC	ACC
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	7.4	700	Clu	C 1 G	Thr>
	c	.c	C	.c	ним	AN T	GG 1	FC T	AG .	, AI Y	wab ~	- GIU	- ren	''''''
									AG	·	·	·	c	c>
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Figure 16

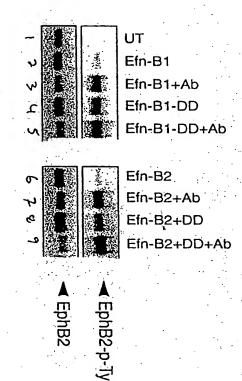


Figure 17

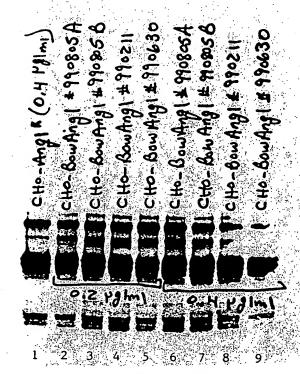
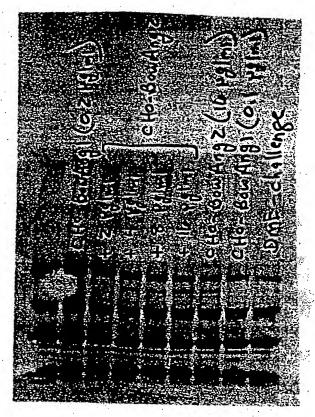


Figure 18



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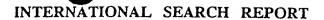




In. .ational Application No PCT/US 99/30900

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C. DOCUM	ENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where appropriate, of the			
	where appropriate, or the	e relevant passages	Relevan	t to claim No.
x	WO 96 37621 A (MORPHOSYS PROTEINOPTIMIERUNG ; PACK PETER	(DE); HOESS	1-5, 12-22	
-	ADOLF (DE)) 28 November 1996 (abstract page 1, line 12 - line 15 page 2, line 4 - line 9 page 14, line 6 - line 11	1996-11-28)		i de la companya de l
(page 16, line 29 - line 34 figure 1A		8-11	
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X Further	documents are listed in the continuation of box C.	χ Patent family members	are listed in annex.	
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5 March 1992 (1992-03-05) abstract page 1 -page 3 page 19, line 14 - line 23 WO 95 27060 A (REGENERON PHARMA) 12 October 1995 (1995-10-12) cited in the application abstract page 23, line 21 -page 24, line 8 claims 4,8 EP 0 816 510 A (TORAY RESEARCH CENTER INC ;TORAY INDUSTRIES (JP)) 7 January 1998 (1998-01-07) abstract DAVIS S. ET AL.: "Isolation of Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning" CELL, vol. 87, 27 December 1996 (1996-12-27), pages 1161-1169, XP002138354 cited in the application the whole document MAISONPIERRE P.C. ET AL.: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355 cited in the application the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"	Category *	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
WO 95 27060 A (REGENERON PHARMA) 12 October 1995 (1995-10-12) cited in the application abstract page 23, line 21 -page 24, line 8 claims 4,8 EP 0 816 510 A (TORAY RESEARCH CENTER INC ;TORAY INDUSTRIES (JP)) 7 January 1998 (1998-01-07) abstract DAVIS S. ET AL.: "Isolation of Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning" CELL, vol. 87, 27 December 1996 (1996-12-27), pages 1161-1169, XP002138354 cited in the application the whole document MAISONPIERE P.C. ET AL.: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355 cited in the application the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"	<i>(</i>	5 March 1992 (1992-03-05) abstract page 1 -page 3	29,32-41 30
EP 0 816 510 A (TORAY RESEARCH CENTER INC; TORAY INDUSTRIES (JP)) 7 January 1998 (1998–01–07) abstract DAVIS S. ET AL.: "Isolation of Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning" CELL, vol. 87, 27 December 1996 (1996–12–27), pages 1161–1169, XP002138354 cited in the application the whole document MAISONPIERRE P.C. ET AL.: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997–07–04), pages 55–60, XP002138355 cited in the application the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"		12 October 1995 (1995-10-12) cited in the application abstract page 23, line 21 -page 24, line 8	
Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning" CELL, vol. 87, 27 December 1996 (1996-12-27), pages 1161-1169, XP002138354 cited in the application the whole document MAISONPIERRE P.C. ET AL.: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355 cited in the application the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"		EP 0 816 510 A (TORAY RESEARCH CENTER INC; TORAY INDUSTRIES (JP)) 7 January 1998 (1998-01-07)	1-22
vol. 87, 27 December 1996 (1996-12-27), pages 1161-1169, XP002138354 cited in the application the whole document MAISONPIERRE P.C. ET AL.: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355 cited in the application the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"		Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning" CELL,	
"Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355 cited in the application the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"		vol. 87, 27 December 1996 (1996-12-27), pages 1161-1169, XP002138354 cited in the application the whole document	
the whole document TOURNAY C. ET AL.: "Uptake of recombinant 21,40 myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms"		"Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355	
activity towards micro-organisms"		the whole document TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to	21,40
DNA CELL BIOLOGY, vol. 15, no. 8, 1996, pages 617-624, XP000907279 abstract page 618		activity towards micro-organisms" DNA CELL BIOLOGY, vol. 15, no. 8, 1996, pages 617-624, XP000907279 abstract	



INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search repor	t	Publication date		Patent family member(s)	Publication t date
WO 9637621	Α	28-11-1996	CA EP JP	2222055 A 0827544 A 11508126 T	28-11-1996 11-03-1998 21-07-1999
WO 9203569	A	05-03-1992	CA EP JP US	2090105 A 0547163 A 6502301 T 5672486 A	01-03-1992 23-06-1993 17-03-1994 30-09-1997
WO 9527060	A	12-10-1995	US AU AU CA EP JP ZA	5747033 A 691915 B 2278995 A 2187167 A 0758381 A 9511401 T 9502762 A	05-05-1998 28-05-1998 23-10-1995 12-10-1995 19-02-1997 18-11-1997 20-02-1996
EP 0816510	Α	07-01-1998	CA WO	2213512 A 9723639 A	03-07-1997 03-07-1997



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23 December 1998 (23.12.98) US

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(74) Agents: PALLADINO, Linda, O.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al. (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

(57) Abstract

Novel fusion polypeptide ligands that bind Eph family receptors or the Tie-2 receptor are identified, and methods for making the fusion polypeptide ligands in biologically active form are described. Nucleic acids encoding these novel fusion polypeptide ligands enable production of the fusion polypeptide ligands. The method of making the nucleic acids and the fusion polypeptide ligands is broadly applicable to the production of polypeptide ligands in general, resulting in improved affinity and/or increased activity of the ligand when compared to its native form.

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METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

This application claims priority of U.S. Application No. 60/113,387, filed December 23, 1998. Throughout this application, various publications are cited. The disclosures of each and all of those publications are hereby incorporated by reference in their entireties into this application.

INTRODUCTION

The present invention provides for novel methods for producing novel fusion polypeptide ligands that have enhanced biological activity as compared to the polypeptide ligands in their native form. The invention also provides for nucleic acids useful for producing biologically active fusion polypeptide ligands, and the fusion polypeptide ligands themselves.

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BACKGROUND OF THE INVENTION

The ability of polypeptide ligands to bind cells and thereby elicit a phenotypic response such as cell growth, survival or differentiation is often mediated through transmembrane tyrosine kinases. The extracellular portion of each receptor tyrosine kinase (RTK) is generally the most distinctive portion of the molecule, as it provides the protein with its ligand-recognizing characteristic. Binding of a ligand to the extracellular domain results in signal transduction via an intracellular tyrosine kinase catalytic domain which transmits a biological signal to intracellular target proteins. The particular array of sequence motifs of this cytoplasmic, catalytic domain determines its access to potential kinase substrates (Mohammadi, et al., 1990, Mol. Cell. Biol., 11: 5068-5078; Fantl, et al., 1992, Cell, 69:413-413).

RTKs appear to undergo dimerization or some related conformational change following ligand binding (Schlessinger, J., 1988, Trend Biochem. Sci.

13:443-447; Ullrich and Schlessinger, 1990, Cell, 61:203-212; Schlessinger and Ullrich, 1992, Neuron 9:383-391); molecular interactions between dimerizing cytoplasmic domains lead to activation of kinase function. In some instances, such as the growth factor platelet derived growth factor (PDGF), the ligand is a dimer that binds two receptor molecules (Hart, et al., 1988, Science, 240: 1529-1531; Heldin, 1989, J. Biol. Chem. 264:8905-8912) while, for example, in the case of EGF, the ligand is a monomer (Weber, et al., 1984, J. Biol. Chem., 259:14631-14636).

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Throughout the history of the biotechnology industry, many novel genes and associated proteins have been identified by virtue of their sequence homology with known genes. Many such proteins are purported to be receptors, but since their cognate ligands have not been identified, they are referred to as orphan receptors. The screening of many of these orphan receptors often leads to the identification of ligands that are capable of binding to the receptor, although the binding is often not associated with activation of any intracellular kinases or any other phenotypic change. Such was the case for members of the Eph receptor family. For sake of clarity, applicants incorporate by reference herein a letter cited as Eph Nomenclature Committee, 1997, published in Cell vol. 90: 403-403 (1997) which sets forth a nomenclature for the Eph Receptor and Eph Ligand Families.

Little, if any, biological activity had been observed in response to binding of a ligand to an Eph family receptor prior to the discovery as set forth in U.S. Patent No. 5,747,033 issued May 5, 1998. That patent describes the concept of "clustering" whereby the soluble domains of ligands were combined to create multimers capable of activating the cognate receptors. Applicants have now extended the concept of clustering to additional ligands outside the Eph family, for example, the Tie-2 receptor ligands known as the angiopoietins, and have also discovered that this method for production of homogeneous forms of clustered ligands is broadly applicable to improve

the affinity and/or increase the activity of a ligand as compared to the native form of the ligand.

Angiopoietin-1 (Ang) is one of two known ligands for the Tie-2 receptor and has been shown to be an agonist for Tie-2 (Davis, et al, 1996, Cell 87:1161-1169), whereas the second known ligand, angiopoietin-2, has been shown to be a naturally occurring antagonist of the Tie-2 receptor (Maisonpierre, et al., 1997, Science 277:55-60). Ang1* is a mutant form of angiopoietin-1 that comprises the N-terminal domain of angiopoietin-2 fused to the coiled-coil domain and the fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. Ang1* has been shown to be a potent agonist for the Tie-2 receptor.

Experiments with mutants of angiopoietin-1 and angiopoietin-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions of, for example Ang-1-FD-Fc, (i.e., the fibrinogen domain of Ang-1 fused to an Fc domain), can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD (dimerization occurs due to the interaction between the Fc components of adjacent molecules). However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD (i.e., the fibrinogen domain of Ang-2) were designed that were intrinsically more highly clustered.

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SUMMARY OF THE INVENTION

The present invention provides for novel, biologically active, soluble forms of polypeptide ligands that bind to receptors on cells. Such polypeptide ligands are useful in promoting a differential function and/or influencing the phenotype, such as growth and/or proliferation, of receptor-bearing

cells. The invention also provides for nucleic acids encoding such polypeptide ligands, and both prokaryotic and eukaryotic expression systems for producing such polypeptide ligands. According to the invention, soluble forms of the polypeptide ligands described herein may be used to promote biological responses in receptor-expressing cells. In particular, a general method is described herein which produces fusion polypeptide ligands that may then be clustered, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

DESCRIPTION OF THE FIGURES

<u>Figure 1A-1E -</u> Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-Fc.

20 <u>Figure 2A-2E</u> - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-FD-Fc.

<u>Figure 3A-3E</u> - Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-Fc-FD.

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<u>Figure 4A-4E</u> - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-Fc-FD.

Figure 5 - Molecular Weight Analysis of Ang-1-FD-Fc-FD protein. SDS

PAGE analyses showing a band running at about 210kD under non-reducing conditions (lane 3) and a band running at about 85kD under reducing conditions (lane 7).

Figure 6 - Light scatter analysis to confirm the molecular weight of Ang-1-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is homogenous.

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Figure 7 - Molecular Weight Analysis of Ang-2-FD-Fc-FD. SDS PAGE analyses showing a band running at about 200kD under non-reducing conditions (lanes 7 and 8) and a band running at about 88kD under reducing conditions (lanes 3 and 4).

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Figure 8 - Light scatter analysis to confirm the molecular weight of Ang-2-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 171kD and presence of a single peak implies that the

protein solution is homogenous.

<u>Figure 9</u> - Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926.

Figure 10 - Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

Figure 11 - Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, at a 20 fold molar excess, angiopoietin-2 is not able to reduce the Ang1*-mediated phosphorylation level to 50%. This result, coupled with the results described in Figure 10 implies that Ang-2-FD-Fc-FD is a more potent inhibitor of Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.

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Figure 12 - Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10.

Figure 13 - Ability of angiopoietin-2 to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at

blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11.

<u>Figure 14A-14E</u> - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B1-Ephrin-B1-Fc.

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<u>Figure 15A-15E</u> - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B2-Ephrin-B2-Fc.

Figure 16 - Comparison of Ephrin-B1-Fc, Ephrin-B1-Ephrin-B1-Fc, Ephrin-B2-Fc and Ephrin-B2-Ephrin-B2-Fc in standard EphB2 phosphorylation assays. COS cells were serum-starved and then left untreated (UT), lane 1, or were treated with unclustered and clustered Ephrin-B1-Fc (Efn-B1), lanes 2 and 3. COS cells were also treated with unclustered and clustered Ephrin-B1-Ephrin-B1-Fc (Efn-B1 DD), lanes 4 and 5. In addition cells were likewise treated with unclustered and clustered Ephrin-B2-Fc (Efn-B2), lanes 6 and 7 and with unclustered and clustered Ephrin-B2-Ephrin-B2-Fc (Efn-B2 DD); lanes 8 and 9. The extent of EphB2 phosphorylation was assessed by anti-phosphotyrosine western blotting (upper panels) and the relative amounts of EphB2 in each lane was determined by anti-EphB2 western blotting (lower panels).

Figure 17 - Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were stimulated with 0.4 μ g/ml Ang1* or 0.2 μ g/ml or 0.4 μ g/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells.

Figure 18 - Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were treated with 0.2

 μ g/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 μ g/ml, 4 μ g/ml, 8 μ g/ml or 16 μ g/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. Ang-2-FD-Fc-FD is able to block or stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

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DETAILED DESCRIPTION OF THE INVENTION

As described in greater detail below, applicants have discovered a method for "clustering" polypeptide ligands, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

The present invention provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

In one embodiment of the invention, the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand. The first and second subunits may each have one or more than one copy of the receptor binding domain of the ligand. In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. Alternatively, the

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receptor binding domain is from a ligand selected from the group consisting of the EPH family of ligands (i.e., the ephrins).

In another embodiment of the invention, the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit. For example, the first subunit may comprise the receptor binding domain of an angiopoietin and the second subunit may comprise the receptor binding domain of vascular endothelial growth factor (VEGF). Alternatively, the first subunit may comprise the receptor binding domain of VEGF and the second subunit may comprise the receptor binding domain an angiopoietin. Still further, the first and second subunits may each have one or more than one copy of the receptor binding domain of their respective ligand.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

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In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(ΔC1).

The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996

and include <u>S. cerevisiae</u> repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) <u>103</u>:321-326); the <u>S. cerevisiae</u> type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. <u>5</u>:3381-3390); the <u>S. calsbergensis</u> alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene <u>36</u>:333-340); and the <u>Neurospora crassa</u> ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. <u>262</u>:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell <u>29</u>:671-679); the <u>S. cerevisiae SUC2</u> gene (Carlson et al., 1983, Mol. Cell. Biol. <u>3</u>:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

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Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as <u>E. coli</u>, a yeast cell, such as <u>Pichia pastoris</u>, an insect cell, such as <u>Spodoptera frugiperda</u>, or a mammalian cell, such as a COS or CHO cell.

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The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

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The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system.

The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

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The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The present invention also provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component. In one embodiment of the invention, the receptor binding domains are fused contiguously. In another embodiment of the invention, the receptor binding domains are from a ligand that is not a member of the EPH family of ligands (i.e., not an ephrin). In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. In an alternative embodiment, the receptor binding domain is from vascular endothelial growth factor (VEGF). In another embodiment, the receptor binding domain is from an ephrin.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

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In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as $Fc(\Delta C1)$.

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The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996 and include S. cerevisiae repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) 103:321-326); the S. cerevisiae type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. 5:3381-3390); the S. calsbergensis alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene 36:333-340); and the Neurospora crassa ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. 262:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); the S. cerevisiae SUC2 gene (Carlson et al., 1983, Mol. Cell. Biol. 3:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is

operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as <u>E. coli</u>, a yeast cell, such as <u>Pichia pastoris</u>, an insect cell, such as <u>Spodoptera frugiperda</u>, or a mammalian cell, such as a COS or CHO cell.

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system. The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

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The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The Examples describe the preparation of novel polypeptide ligands that comprise a receptor binding domain of a member of the Eph (Eph transmembrane tyrosine kinase family ligands) family of ligands or of a

member of the angiopoietin family of ligands that can bind the Tie-2 receptor.

For a description of novel Eph family ligands, methods of making and using them, as well as the sequences of EHK-1L, B61 and ELK-L, together with a description of a method of enhancing the biological activity of EPH family ligands by clustering them, applicants refer to U.S. Patent No. 5,747,033 issued on May 5, 1998 which is hereby incorporated by reference in its entirety. Applicants further refer to International Application PCT/US93/10879, published as WO 94/11020 on May 26, 1994; and International Application PCT/US96/17201 published as WO 97/15667 entitled "Biologically Active EPH Family Ligands" each of which is hereby incorporated by reference in its entirety.

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As has been previously reported, a family of ligands for the TIE-2 receptor has been discovered and named the Angiopoietins. This family, consisting of TIE-2 ligand 1 (Ang-1); TIE-2 ligand 2 (Ang-2); TIE ligand 3 (Ang-3); and TIE ligand 4 (Ang-4) has been extensively characterized. For a description of the cloning, sequencing and characterization of the angiopoietins, as well as for methods of making and uses thereof, including the production and characterization of modified and chimeric ligands thereof, reference is hereby made to the following publications, each of which is incorporated by reference herein in its entirety: U.S. Patent No. 5,521,073 issued May 28, 1996; U.S. Patent No. 5,643,755 issued July 1, 1997; U.S. Patent No. 5,650,490 issued July 22, 1997; U.S. Patent No. 5,814,464 issued September 29, 1998; U.S. Patent No. 5,879,672 issued March 9, 1999; U.S. Patent No. 5,851,797 issued December 22, 1998; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof," published as WO 96/11269 on 18 April 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof,"published as WO 96/31598 on 10 October 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled

"TIE-2 Receptor Ligands (TIE Ligand-3; TIE Ligand-4) And Their Uses," published as WO 97/48804 on 24 December 1997 in the name of Regeneron Pharmaceuticals, Inc; and PCT International Application entitled "Modified TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc.

When used herein, fusion polypeptide includes functionally equivalent molecules in which amino acid residues are substituted for residues within the sequence resulting in a silent or conservative change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent or conservative alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar 15 (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular

Cells that express the fusion polypeptides of the invention are genetically engineered to produce them by, for example, transfection, transduction, electroporation, or microinjection.

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The present invention encompasses the nucleic acid sequences encoding the fusion polypeptides of the invention, as well as sequences that hybridize under stringent conditions to nucleic acid sequences that are complementary to the nucleic acid sequences of the invention. Stringent conditions are set forth in, for example, Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). In addition, the present invention encompasses nucleic acid sequences that are different from the nucleic acid sequences of the invention but which nevertheless encode the fusion polypeptides of the invention due to the degeneracy of the genetic code.

In addition, the present invention contemplates use of the fusion polypeptides described herein in tagged forms.

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Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion polypeptides of the invention using appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of nucleic acid sequence encoding the fusion polypeptides of the invention may be regulated by a second nucleic acid sequence so that the fusion polypeptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the fusion polypeptides described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the fusion polypeptide include, but are not limited to the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980,

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Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the b-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. <u>75</u>:3727-3731), or the <u>tac</u> promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell <u>38</u>:647-658; Adames et al., 1985, Nature <u>318</u>:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene

control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising Eph fusion polypeptide encoding or angiopoietin fusion polypeptide encoding nucleic acids as described herein, are used to transfect the host and thereby direct expression of such nucleic acid to produce fusion polypeptides which may then be recovered in biologically active form. As used herein, a biologically active form includes a form capable of binding to the relevant receptor and causing a differentiated function and/or influencing the phenotype of the cell expressing the receptor. Such biologically active forms would, for example, induce phosphorylation of the tyrosine kinase domain of the Ehk-1, Elk, or Tie2 receptor, or stimulation of synthesis of cellular DNA.

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Expression vectors containing the nucleic acid inserts can be identified by three general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign nucleic acids inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted nucleic acid sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign nucleic acid sequences in the vector. For example, if an efl nucleic acid sequence is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign nucleic acid product expressed by the recombinant. Such assays can be based, for example, on the physical or

functional properties of the nucleic acid product of interest, for example, by binding of a ligand to a receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or binding to antibodies produced against the protein of interest or a portion thereof.

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Cells of the present invention may transiently or, preferably, constitutively and permanently express the ephrin or angiopoietin fusion polypeptide as described herein.

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The ephrin fusion polypeptides of the invention may be useful in methods of treating a patient suffering from a neurological disorder comprising treating the patient with an effective amount of the ephrin fusion polypeptide.

For example, the Elk receptor is expressed primarily in brain. Accordingly, it is believed that an Elk binding ephrin fusion polypeptide ligand will support the induction of a differential function and/or influence the phenotype, such as growth and/or survival of neural cells that express this receptor.

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The present invention also provides for pharmaceutical compositions comprising the ephrin fusion polypeptide in a suitable pharmacologic carrier. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

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As our understanding of neurodegenerative disease/neurotrauma becomes clearer, it may become apparent that it would be beneficial to decrease the effect of endogenous Efl-6. Therefore, in areas of nervous system trauma, it may be desirable to provide Efl-6 antagonists, including, but not limited to,

fusion polypeptide forms of Efl-6 which may compete with cell-bound ligand for interaction with Elk receptor. It may be desirable to provide such antagonists locally at the injury site rather than systemically. Use of an Efl-6 antagonist providing implant may be desirable.

Alternatively, certain conditions may benefit from an increase in Efl-6 responsiveness. It may therefore be beneficial to increase the number or binding affinity of Efl-6 in patients suffering from such conditions.

The invention herein further provides for the development of a fusion polypeptide, as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE-2 receptor. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

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Because TIE-2 receptor has been identified in association with endothelial cells and, as was previously demonstrated, blocking of agonists of the receptor such as TIE-2 ligand 1 (Ang-1) has been shown to prevent vascularization, applicants expect that TIE-2 agonist fusion polypeptides of the invention may be useful for the induction of vascularization in diseases or disorders where such vascularization is indicated. Such diseases or disorders would include wound healing, ischemia and diabetes. The ligands may be tested in animal models and used therapeutically as described for other agents, such as vascular endothelial growth factor (VEGF), another endothelial cell-specific factor that is angiogenic. Ferrara, et al. U.S. Patent No. 5,332,671 issued July 26, 1994. The Ferrara reference, as well as other studies, describe in vitro and in vivo studies that may be used to demonstrate the effect of an angiogenic factor in enhancing blood flow to ischemic myocardium, enhancing wound healing, and in other therapeutic

Patent Application 0 550 296 A2 published July 7, 1993; Banai, et al. Circulation 89:2183-2189 (1994); Unger, et al. Am. J. Physiol. 266:H1588-H1595

settings wherein neoangiogenesis is desired. [see Sudo, et al., European

(1994); Lazarous, et al. Circulation 91:145-153 (1995)]. According to the invention, the agonist fusion polypeptides may be used alone or in combination with one or more additional pharmaceutically active compounds such as, for example, VEGF or basic fibroblast growth factor (bFGF).

Conversely, antagonists of the TIE-2 receptor, such as TIE-2 receptorbodies or TIE-2 ligand 2 (Ang-2) as described in Example 9 in International Publication No. WO 96/31598 published 10 October 1996, have been shown to prevent or attenuate vascularization, and are thus expected to be useful in preventing or attenuating, for example, tumor growth. Similarly then, TIE-2 antagonist fusion polypeptides of the invention would also be useful for those purposes. These antagonists may be used alone or in combination with other compositions, such as anti-VEGF antibodies, that have been shown to be useful in treating conditions in which the therapeutic intent is to block angiogenesis.

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For example, applicants have determined that TIE-2 ligands are expressed in cells within, or closely associated with, tumors. For example, TIE-2 ligand 2 (Ang-2) appears to be tightly associated with tumor endothelial cells. Accordingly, TIE-2 antagonist fusion polypeptides of the invention may also be useful in preventing or attenuating, for example, tumor growth.

In other embodiments, the TIE-2 agonist fusion polypeptides of the invention described herein may be used as hematopoietic factors. A variety of hematopoietic factors and their receptors are involved in the proliferation and/or differentiation and/or migration of the various cells types contained within blood. Because the TIE-2 receptors are expressed in early hematopoietic cells, the TIE-2 ligands are expected to play a comparable role in the proliferation or differentiation or migration of these cells. Thus, for example, TIE-2 agonist fusion polypeptide compositions may be prepared, assayed, examined in in vitro and in vivo biological systems and

used therapeutically as described in any of the following: Sousa, U.S. Patent No. 4,810,643, Lee, et al., Proc. Natl. Acad. Sci. USA 82:4360-4364 (1985) Wong, et al. Science, 228:810-814 (1985); Yokota, et al. Proc. Natl. Acad. Sci (USA) 81:1070 (1984); Bosselman, et al. WO 9105795 published May 2, 1991 entitled "Stem Cell Factor" and Kirkness, et al. WO 95/19985 published July 27, 1995 entitled "Haemopoietic Maturation Factor". Accordingly, the fusion polypeptides may be used to diagnose or treat conditions in which normal hematopoiesis is suppressed, including, but not limited to anemia, thrombocytopenia, leukopenia and granulocytopenia. In a preferred embodiment, the fusion polypeptides may be used to stimulate differentiation of blood cell precursors in situations where a patient has a disease, such as acquired immune deficiency syndrome (AIDS) which has caused a reduction in normal blood cell levels, or in clinical settings in which enhancement of hematopoietic populations is desired, such as in conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment or chemotherapy.

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The fusion polypeptides of the present invention may be used alone, or in combination with another pharmaceutically active agents such as, for example, cytokines, neurotrophins, interleukins, etc. In a preferred embodiment, the fusion polypeptides may be used in conjunction with any of a number of factors which are known to induce stem cell or other hematopoietic precursor proliferation, or factors acting on later cells in the hematopoietic pathway; including, but not limited to, hemopoietic maturation factor, thrombopoietin, stem cell factor, erythropoietin, G-CSF, GM-CSF, etc.

In an alternative embodiment, TIE-2 receptor antagonist fusion polypeptides are used to diagnose or treat patients in which the desired result is inhibition of a hematopoietic pathway, such as for the treatment of myeloproliferative or other proliferative disorders of blood forming organs

such as thrombocythemias, polycythemias and leukemias. In such embodiments, treatment may comprise use of a therapeutically effective amount of the fusion polypeptides as described herein.

Effective doses useful for treating these or other diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975)]. Pharmaceutical compositions for use according to the invention include the fusion polypeptides described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation prior to administration in vivo. For example, the pharmaceutical composition may comprise a fusion polypeptide in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

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Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

The present invention also provides for pharmaceutical compositions comprising the fusion polypeptides described herein, in a pharmacologically acceptable vehicle. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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<u>EXAMPLES</u>

Angiopoietin ligands:

As described *supra*, experiments with mutants of Ang-1 and Ang-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions (dimerization occurs due to the interaction between the Fc components of adjacent molecules), for example Ang-1-FD-Fc, can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD. However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD were designed that were intrinsically more highly clustered.

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Two general types of nucleic acid molecules were constructed. The first type consisted of two tandem copies of Ang-1-FD fused to an Fc tag, thus leading

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to a secreted polypeptide molecule that is dimeric with respect to the Fc tag but tetrameric with respect to Ang-1-FD. Similarly, two tandem copies of Ang-2-FD fused to an Fc tag constituted the angiopoietin-2 version of this type of construct. These molecules were designated Ang-1-FD-FD-Fc and Ang-2-FD-FD-Fc, respectively.

In the second type of nucleic acid molecule constructed, two copies of Ang-1-FD were connected by an Fc tag bridging between them, thus creating the structure Ang-1-FD-Fc-FD that is still dimeric with respect to the Fc, as well as tetrameric with respect to Ang-1-FD. An angiopoietin-2 version was also constructed and these two molecules were designated Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD, respectively.

For either type of construct, similar properties were observed: unlike dimeric Ang-1-FD-Fc, which fails to activate Tie-2 in endothelial cells, both Ang-1-FD-Fc and Ang-1-FD-Fc-FD could readily activate Tie-2 in endothelial cells, with a potency comparable to that of the native ligand. Also, like native angiopoietin-2, Ang-2-FD-Fc-FD could antagonize angiopoietin-1 activity with a potency that is comparable to that of native angiopoietin-2, and with much greater potency than the marginally antagonistic activity of the Ang-2-FD-Fc dimer.

Construction of mutant angiopoietin nucleic acid molecules.

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All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into the mammalian expression vector pMT21 (Genetics

Institute, Inc.) with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res: 15:8125-8148) at the 5' end to promote protein translation. The bridging sequences described *infra* were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

Example 1: Construction of the Ang-1-FD-Fc, Ang-2-FD-Fc, Ang-1-FD-Fc-FD, and Ang-2-FD-Fc-FD nucleic acid molecules.

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Ang-1-FD-Fc: Ang-1-FD-Fc consists of a trypsin signal sequence at its amino terminus to allow for secretion (bases 1-45 of Figure 1A) followed by the angiopoietin-1 fibrinogen domain (FD) (bases 46-690 of Figure 1A-Figure 1B), a short bridging sequence consisting of the amino acids Gly-Pro Ala-Pro (bases 691-702 of Figure 1B), a second angiopoietin-1 FD (bases 703-1750 of Figure 1B-Figure 1D), another bridging sequence consisting of the amino acids Gly-Pro-Gly (bases 1351-1359 of Figure 1D), and the coding sequence for the Fc portion of human IgG1 (bases 1360-2058 of Figure 1D-Figure 1E).

Ang-2-FD-FC: The Ang-2-FD-FC nucleic acid molecule was similarly constructed. It consists of a trypsin signal sequence (bases 1-45 of Figure 2A), an angiopoietin-2 FD (bases 46-690 of Figure 2A- Figure 2B), a bridging amino acid sequence Gly-Gly-Pro-Ala-Pro (bases 691-705 of Figure 2B), a second angiopoietin-2 FD (bases 706-1353 of Figure 2B-Figure 2D), another bridging amino acid sequence Gly-Pro-Gly (bases 1354-1362 of Figure 2D), and the coding sequence for the Fc portion of human IgG1 (bases 1363-2061 of Figure 2D-Figure 2E.

Ang-1-FD-Fc-FD: The Ang-1-FD-Fc-FD consists of a trypsin signal sequence (bases 1-45 of Figure 3A), an angiopoietin-1 FD (bases 46-690 of Figure 3A-3B), the bridging amino acid sequence Gly-Pro-Gly (bases 691-699 of Figure

3B), the coding sequence for the Fc portion of human IgG1 (bases 700-1395 of Figure 3B-3D), another bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1396-1419 of Figure 3D), and a second angiopoietin-1 FD (bases 1420-2067 of Figure 3D-Figure 3E).

Ang-2-FD-Fc-FD: The Ang-2-FD-Fc-FD nucleic acid molecule consists of a trypsin signal sequence (bases 1-45 of Figure 4A), an angiopoietin-2 FD domain (bases 46-690 of Figure 4A-Figure 4B), the bridging amino acid sequence Gly-Gly-Pro-Gly (bases 691-702 of Figure 4B), the coding sequence for the Fc portion of human IgG1 (bases 703-1398 of Figure 4B- Figure 4D), the bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1399-1422 of Figure 4D), and a second angiopoietin-2 FD (bases 1423-2067 of Figure 4D-Figure 4E).

Example 2: Characterization of Ang-1 FD-Fc-FD protein.

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Molecular Weight Analysis: The predicted molecular weight for Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses of COS cell-derived protein described infra confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions (Figure 5). Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To

determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. Figure 6 shows the results of this analysis using COS cell-derived protein. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is, in fact, homogenous.

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Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang1-FD-Fc-FD DNA construct described supra. All transfections were performed using standard techniques known in the art. The COS cell supernatant was analyzed using Biacore technology (Pharmacia, Inc.) to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 765, which is equivalent to 0.9mg of recombinant protein/liter of COS cell supernatant. These values represent very high levels of expression.

Purification of COS Supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). In fact, the relative ease of purification of the Ang-1-FD-Fc-FD protein gives it a distinct advantage over the parent protein, angiopoietin-1, from which

it is derived, and the mutant version of angiopoietin-1 called Ang1* that consists of the N-terminal of angiopoietin-2 fused to the coiled-coil domain and fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. (See PCT International Application entitled "Modified TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc., especially Figure 27, which is hereby incorporated by reference).

Both angiopoietin-1 and Ang1* require extensive, expensive and labor-intensive purification schemes that result in relatively poor yields of recombinant protein. The need for cost-effective, simple purification schemes for biologicals intended for clinical use can not be over-emphasized.

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The COS cell supernatant was purified as described *supra* and yielded approximately 1 mg of purified Ang-1-FD-Fc-FD protein that was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of COS cell-derived Ang-1-FD-Fc-FD protein:

Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. This was of concern because the mutant molecule, Ang1*, has a history of containing between 10-20% N-terminally truncated species. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

Receptor binding analysis of COS cell-derived Ang-1-FD-Fc-FD: Previous studies have determined that the fibrinogen domain (FD) of the angiopoietin molecules is necessary for ligand/receptor interaction. Furthermore, in order for high affinity binding to the Tie-2 receptor to

occur, native angiopoietin-1, angiopoietin-2, and the mutant Ang1* must form at least tetrameric, and possibly higher order, multimers. To determine whether the COS cell-derived Ang-1-FD-Fc-FD protein, which is tetrameric with respect to the FD domain, could bind to Tie-2 with high affinity, standard Biacore analysis was performed. Briefly, Tie-2-Fc receptor body protein, which is a fusion protein comprising the ectodomain of Tie-2 fused to the Fc domain of human IgG1, was immobilized on a Biacore chip. Ang-1-FD-Fc-FD-containing solution was passed over the chip and binding between Tie-2 ectodomain and Ang-1-FD-Fc-FD was allowed to occur. The binding step was followed by a 0.5 M NaCl high salt wash. The high salt wash was not able to disrupt the interaction between the Ang-1-FD-Fc-FD protein and the Tie-2 receptor ectodomain, implying that there is a strong interaction between the mutant ligand and receptor. This result is consistent with earlier Biacore results in which both Ang-1-FD-Fc-FD parent molecule, angiopoietin-1 and the mutant Ang1* molecule, have been shown to interact strongly with the Tie-2-Fc receptor and that this interaction is not disrupted by high salt. In contrast, several mutant molecules derived from the parent angiopoietin-1 molecule are readily dissociated from the Tie-2-Fc receptor when treated with high salt. The mutant molecules, designated Ang-1/FD (a monomer with respect to the FD), Ang-1/FD-Fc (also a monomer with respect to the FD, but which is able to form a dimer due to the presence of the Fc domain), and Ang-1/C/FD (a monomer with respect to the FD, but which also contains the coiled-coil domain of angiopoietin-1), do not exist in multimeric forms sufficient for high affinity binding to the Tie-2 receptor.

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Example 3: Characterization of COS cell-derived Ang-2-FD-Fc-FD protein.

Molecular Weight Analysis: As described for Ang-1-FD-Fc-FD supra, the predicted molecular weight for Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form of Ang-2-FD-Fc-FD has a predicted

weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104. SDS PAGE analyses of COS cell-derived protein confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 88kD under reducing

conditions (Figure 7). Light scatter analysis confirmed the molecular weight

(171kD) and revealed that the Ang-2-FD-Fc-FD protein, like Ang-1-FD-Fc-

10 FD, exists as a homogeneous species (Figure 8).

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang-2-FD-Fc-FD DNA construct described supra. The COS cell supernatant was analyzed by Biacore to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 606, which is equivalent to 0.7mg of recombinant protein/liter of COS cell supernatant. These values represent relatively high levels of expression.

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Purification of COS Supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The COS cell supernatant was purified as described for Ang-1-FD-Fc-FD supra and yielded approximately 2 mg of purified Ang-2-FD-Fc-FD protein that was used in the studies described *infra* to further characterize this protein.

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N-terminal sequencing: Purified COS cell-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Glu, wherein X is Cys. This

sequence can be found at amino acids 16-20 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Receptor binding analysis of COS cell-derived protein: To determine whether the COS cell-derived Ang-2-FD-Fc-FD protein could bind to the Tie-2 receptor, standard Biacore analysis was performed as described for Ang-1-FD-Fc-FD supra. As with Ang-1-FD-Fc-FD, a high salt wash was not able to disrupt the interaction between the Ang-2-FD-Fc-FD protein and the Tie-2-Fc receptor, again implying that there is a strong interaction between mutant ligand and receptor.

Example 4: Effects of COS cell-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

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Because Ang-1-FD-Fc-FD is a mutant molecule derived from the agonist angiopoietin-1 and Ang-2-FD-Fc-FD is a mutant molecule derived from the antagonist angiopoietin-2, we wanted to determine whether or not these two mutant molecules would retain the activity associated with the parent molecule from which it was derived.

Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell; C. J.; et al., (1983) Proc. Natl. Acad. Sci. USA <u>80</u>:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

(A) Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with either 0.1 μg/ml, 0.2 μg/ml, or 0.8 μg/ml Ang1* or Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 9).

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- (B) Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.4 μ g/ml of the Tie-2 agonist Ang1* and 1 μ g/ml, 2 μ g/ml, 4 μ g/ml. 6 μ g/ ml, or 8 μ g/ml of Ang-2-FD-Fc-FD. As shown in Figure 10, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.
- (C) Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells: To compare the blocking effects of the naturally occurring antagonist angiopoietin-2 with that of Ang-2-FD-Fc-FD, the same experiment described in (B) *supra* was performed, substituting angiopoietin-2 for Ang-2-FD-Fc-FD. The results of this experiment are shown in Figure 11. At a 20 fold molar excess, the angiopoietin-2 has not reduced the phosphorylation level to 50%. This result, coupled with the results described in (B) *supra* implies that Ang-2-FD-Fc-FD is a more potent inhibitor or Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.
- 20 (D) Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the naturally occurring Tie-2 agonist angiopoietin-1 and 1 μg/ml, 2 μg/ml, 4 μg/ml. 6 μg/ ml, or 8 μg/ml of Ang-2-FD-Fc-FD. The results of this experiment, shown in Figure 12, show that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10 and described in (B) supra.
- (E) Ability of angiopoietin-2 to block angiopoietin-1-mediated

 phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the angiopoietin-1 and 1 μg/ml, 2 μg/ml, 4 μg/ml,

6 μg/ ml, or 8 μg/ml of angiopoietin-2. The results of this experiment, shown in Figure 13, show that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11 and described in (C) *supra*.

Example 5: Construction of Ang-1-FD-Fc-FD CHO cell expression vector pRG763/Ang-1-FD-Fc-FD.

The pRG763/Ang-1-FD-Fc-FD CHO cell expression vector was constructed by isolating from the plasmid pCDNA3.1/Ang1-FD-Fc-FD a 2115 base pair EcoRI - NotI fragment containing Ang1-FD-Fc-FD and ligating this fragment into pRG763 vector digested with EcoRI and NotI. A large scale (2L) culture of E. coli DH10B cells carrying the pRG763/Ang-1-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was verified by digestion of aliquots with NcoI and HincII restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

Example 6: Expression of Ang-1-FD-Fc-FD in CHO cells.

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Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of pRG763/Ang-1-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10%

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FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *infra*.

Example 7: Construction of Ang-2-FD-Fc-FD CHO cell expression vector pRG763/Ang-2-FD-Fc-FD.

The plasmid pRG763/Ang-2-FD-Fc-FD was constructed by isolating from the plasmid pCDNA3.1/Ang-2-FD-Fc-FD a 2097 base pair EcoRI - NotI fragment containing Ang-2-FD-Fc-FD and ligating this fragment into the pRG763 vector digested with EcoRI and NotI. A large scale (1L) culture of E. coli DH10B cells carrying the pRG763/Ang-2-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of plasmid DNA with NcoI and Ppu10I restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

25 Example 8: Expression of Ang-2-FD-Fc-FD in CHO cells.

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Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of pRG763/Ang-2-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after

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adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles purified as described infra.

10 Example 9: Characterization of stable CHO clone-derived Ang-1-FD-Fc-FD protein.

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Molecular Weight Analysis: The predicted molecular weight for stable CHO clone-derived Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology

Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. The results of this analysis show that the dimeric protein appears to be approximately 173.9kD and the presence of a single peak implies that the protein solution is homogenous.

Expression level of Ang-1-FD-Fc-FD in stable CHO clones: CHO cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-1-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of 2-3 pg/cell/day.

Purification of Ang-1-FD-Fc-FD protein derived from stable CHO clone supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). The CHO cell supernatant was purified as described supra and the purified ANG-1-FD-Fc-FD protein was used in the studies described infra to further characterize the protein.

N-terminal sequencing of stable CHO clone-derived Ang-1-FD-Fc-FD protein: Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal

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sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

Example 10: Characterization of stable CHO clone-derived Ang-2-FD-Fc-FD protein.

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Molecular Weight Analysis: As described for stable CHO clone-derived Ang-1-FD-Fc-FD supra, the predicted molecular weight for stable CHO clone-derived Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT). The monomeric form of Ang-2-FD-Fc-FD has a predicted weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104. SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis confirmed the molecular weight (176.6kD) and revealed that the stable CHO clonederived Ang-2-FD-Fc-FD protein, like stable CHO clone-derived Ang-1-FD-Fc-FD, exists as a homogeneous species.

Expression level of Ang-2-FD-Fc-FD derived from stable CHO clones: CHO cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-2-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of approximately 1-2 pg/cell/day.

Purification of stable CHO clone-derived Ang-2-FD-Fc-FD from cell supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The CHO cell supernatant was purified as described for stable CHO clone-derived Ang-1-FD-Fc-FD supra and was used in the studies described infra to further characterize this protein.

N-terminal sequencing of stable CHO clone-derived Ang-2-FD-Fc-FD protein: Purified stable CHO clone-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Asp-X-Ala-Glu-Val, wherein X is Cys. This sequence can be found at amino acids 17-21 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Example 11: Effects of stable CHO clone-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

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Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA <u>80</u>:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

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(A) Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with 0.4 μg/ml Ang1* or 0.2 μg/ml or 0.4 μg/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that or stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 17).

(B) Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.2 μ g/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 μ g/ml, 4 μ g/ml, 8 μ g/ml or 16 μ g/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. As shown in Figure 18, Ang-2-FD-Fc-FD is able to block stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

Ephrin ligands:

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In previous experiments (Davis et al., 1994, Science, 266:816-819; Gale et al., 1996, Neuron 17:9-19, Gale and Yancopoulos, 1997, Cell Tissue Research 290:227-241), soluble, unclustered Ephrin-B1-Fc and Ephrin-B2-Fc, which dimerize at their respective Fc domains and therefore are dimeric with respect to either the Ephrin-B1 or Ephrin-B2 ectodomain, failed to induce EphB2 receptor phosphorylation. However, when either molecule was multimerized by pre-clustering with an anti-Fc antibody, they became potent agonists for the EphB2 receptor, as judged by tyrosine phosphorylation of the EphB2 receptor in a COS cell reporter assay. Because multimerization of both Ephrin-B1 and Ephrin-B2 appears to be necessary for induction of receptor phosphorylation, we theorized that a molecule that contained tandem repeats of either Ephrin-B1 or Ephrin-B2 ectodomains fused to an Fc domain, which would be dimeric with respect to the Fc domain but which would be tetrameric with respect to Ephrin ectodomains, might be sufficiently clustered to induce receptor phosphorylation. To test this hypothesis, the following DNA constructs were constructed, recombinant proteins produced, and reporter assays performed.

Construction of tandem Ephrin ectodomain/Fc domain nucleic acid molecules.

All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into either the mammalian expression pJFE14 (Ephrin-B1-Ephrin-B1-Fc) or pMT21 (Ephrin-B2-Ephrin-B2-Fc), each with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5' end to promote protein translation. The bridging sequences described infra were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

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Example 12: Construction of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules.

(A) Ephrin-B1-Ephrin-B1-Fc: The Ephrin-B1-Ephrin-B1-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B1 (Davis et al., ibid.), which corresponds to nucleotides 1-711 of Figure 14A-Figure 14B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 712-720 of Figure 14B), followed by a second copy of the ectodomain of Ephrin-B1 (corresponding to nucleotides 721-1344 of Figure 14B-Figure 14D), except that in this copy of the Ephrin-B1 ectodomain the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1345-1353 of Figure 14D),

followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1354-2049 of Figure 14D-Figure 14E).

(B) Ephrin-B2-Ephrin-B2-Fc: The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E).

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As with the angiopoietin nucleic acid molecules described *supra*, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

20 Example 13: Expression of tandem Ephrin recombinant proteins in COS cells.

COS cells were transiently transfected with either the Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules described *supra* using standard transfection techniques known in the art. Two days subsequent to transfection, the growth medium (DMEM supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 10% calf serum) was aspirated and replaced with serum-free medium (DMEM supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine). Cell were grown for an additional three days and then the serum-free medium containing the recombinant proteins was collected. Recombinant protein concentration was determined by performing dot blots and comparing the

signal obtained to a standard curve. Once approximate protein concentrations were determined, the Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc recombinant proteins were used in the cell reporter assays described *infra*.

Example 14: Characterization of the COS cell-derived tandem Ephrin ectodomain/Fc domain recombinant proteins.

Reporter Assay: COS cells, which endogenously express the Eph family receptor EphB2 (Gale et al., 1996, Neuron 17:9-19), were used in reporter assays to evaluate the ability of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc to induce receptor phosphorylation. The assays were performed as previously described (Davis et al., ibid.; Gale et al., ibid.). Briefly, COS cells were grown to 80-90% confluency in standard growth medium described supra. After growth, the medium was aspirated, and replaced with serum-free medium (described supra) for 1-2 hours prior to treatment with either Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc recombinant protein. The cells were stimulated with 500 ng/ml Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc for 30 minutes at 37°C, with or without affinity purified human IgG1 Fc-specific goat anti-human antibody (Jackson Immunoresearch, West Grove, PA) at a final concentration of 17 µg/ml. This antibody is capable of clustering the Fc tagged fusion. Subsequent to treatment, the COS cells were harvested and cell lysates were prepared as described in Davis, et al. and Gale, et al., supra. The EphB2 receptor protein was immunoprecipitated from the cell lysates using an anti-EphB2 antisera (Henkemeyer et al., 1994, Oncogene 2:1001-1014). Immunoprecipitates were resolved by standard SDS PAGE and transferred to PVDF membranes (Millipore) for western blot analysis. The membranes were probed with either anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology Institute, Lake Placid, NY) or anti-EphB2 antibodies (Henkemeyer, et al., ibid.) to determine the extent of EphB2

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phosphorylation and the relative quantities of EphB2 in the experimental conditions described *supra*.

Results: Both Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc were shown to be approximately as active as anti-Fc antibody-clustered Ephrin-B1-Fc in their ability to induce EphB2 receptor phosphorylation in the COS cell reporter assay. Furthermore, if either of the proteins were further clustered with the goat anti-human Fc antibody, they became even more potent in their ability to induce EphB2 receptor phosphorylation. Figure 16 shows the results of this phosphorylation assay.

Example 15: Construction of Ephrin-B2-Ephrin-B2-Fc CHO expression vector.

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The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E). This molecule was subcloned into the HindIII and NotI polylinker sites in the expression vector pRG763 and was designated pRG763-m(Ephrin-B2)2-Fc. As with the angiopoietin nucleic acid molecules described supra, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

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Example 16: Expression of Ephrin-B2-Ephrin-B2-Fc in CHO-K1 (E1A) cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 106 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 µg of pRG763-m(Ephrin-B2)2-Fc using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *supra*.

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William Contract

WHAT IS CLAIMED IS:

- 1. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.
- 2. The nucleic acid of claim 1, wherein the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand.
- 3. The nucleic acid of claim 1, wherein the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit.
- 4. The nucleic acid of claim 2, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.
- 5. The nucleic acid of claim 3, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.
- 6. The nucleic acid of claim 2, wherein the receptor binding domain is the fibringen domain of angiopoietin-1 or angiopoietin-2.
- 7. The nucleic acid of claim 4, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

8. The nucleic acid of claim 2, wherein the ligand is selected from the group consisting of the EPH family of ligands.

- 9. The nucleic acid of claim 4, wherein the ligand is selected from the group consisting of the EPH family of ligands.
- 10. The nucleic acid of claims 1 through 9, wherein the multimerizing component comprises an immunoglobulin derived domain.
- 11. The nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
- 12. A fusion polypeptide encoded by the nucleic acid molecule of claims 1 through 11.
- 13. A composition comprising a multimer of the fusion polypeptide of claim 12.
- 14. The composition of claim 13, wherein the multimer is a dimer.
- 15. A vector which comprises the nucleic acid molecule of claims 1 through 11.
- 16. An expression vector comprising a nucleic acid molecule of claims 1 through 11, wherein the nucleic acid molecule is operatively linked to an expression control sequence.
- 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.

18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

- 19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.
- 20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.
- 21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.
- 22. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 17 through 21, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.
- 23. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component.
- 24. The nucleic acid of claim 23, wherein the receptor binding domains are fused contiguously.
- 25. The nucleic acid of claim 23, wherein the ligand is not a member of the EPH family of ligands.
- 26. The nucleic acid of claim 24, wherein the ligand is not a member of

the EPH family of ligands.

27. The nucleic acid of claim 23, wherein the receptor binding domain is the fibringen domain of angiopoietin-1 or angiopoietin-2.

- 28. The nucleic acid of claim 24, wherein the receptor binding domain is the fibringen domain of angiopoietin-1 or angiopoietin-2.
- 29. The nucleic acid of claims 23 through 28, wherein the multimerizing component comprises an immunoglobulin derived domain.
- 30. The nucleic acid molecule of claim 29, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
- 31. A fusion polypeptide encoded by the nucleic acid molecule of claims 23 through 30.
- 32. A composition comprising a multimer of the fusion polypeptide of claim 31.
- 33. The composition of claim 32, wherein the multimer is a dimer.
- 34. A vector which comprises the nucleic acid molecule of claims 23 through 30.
- 35. An expression vector comprising a nucleic acid molecule of claims 23 through 30, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

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36. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 35, in a suitable host cell.

- 37. The host-vector system of claim 36, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.
- 38. The host-vector system of claim 36, wherein the suitable host cell is E. coli.
- 39. The host-vector system of claim 36, wherein the suitable host cell is a COS cell.
- 40. The host-vector system of claim 36, wherein the suitable host cell is a CHO cell.
- 41. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 36 through 40, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

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Fia.1C. 870 880 890 900 AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe> _d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__d_ 910 920 930 940 GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe> _d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__ 950 960 970 990 GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met> _d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__> 1000 1010 1030 GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His> 1040 1050 1080 ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His> d__d__d__ang1 fibrinogen-like domain_d__d_ 1090 1120 1100 1110 ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT GCT Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala> _d__d__angi fibrinogen-like domain_d__d__d__d 1130 1140... GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys> _d__ang1 fibrinogen-like domain_d__d__d__d__d__ 1190 1210 TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly> _d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d_ 1220 1230 1250 1260 CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT Pro Ser Ash Leu Ash Gly Met Phe Tyr Thr Ala Gly Gln Ash His> _d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__d__d__d__>

Fig.1D. 1280 1300 GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC AGT Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser> _d__d__d__ANG1 FIBRINOGEN-LIKE DOMAIN_d__ _d__ 1310 1320 1330 1350 TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe> d_d_d_ANG1 FIBRINOGEN-LIKE DOMAIN_d_d_d_d_> 1360 1370 1380 1390 GGA CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA Gly Pro Gly> ___e___e__ Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro> _f___f__FC TAG [SPLIT]_f___f__f__ 1400 1430 CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu> _f___f__f__f__f_ TAG [SPLIT]___f__f_ 1460 1470 TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro> _f__f__f__f_ TAG [SPLIT]___f__ 1490 1500 GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu> f_f_f_rc tag [SPLIT]__f_f__f_ GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala> _f__fC TAG (SPLIT)___f_ _f__f__ 1590 1610 AAG ACA AAG CCG CGG GAG GAG CAG TAC AGC ACG TAC CGT GTG Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val> _f___f__f__f__FC TAG [SPLIT]___f___f__f_ 1630 1640 1650 GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys> _f__f__f__FC TAG (SPLIT)___f__f__f__f_____

5/38 1680 1690 1700 1710 GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile> _f___f__f__f__FC TAG (SPLIT)___f__f__f__f_______ 1720 1730 1750 GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln> ___f__f__f__f__f__FC TAG [SPLIT]__f__f__f__f_____> 1760 1770 1790 1800 GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln> _f__f__f__f__f__FC TAG [SPLIT]___f__f__f_____> 1820 1840 GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile> 1860 1870 1850 GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys> 1900 ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr> Carrier Carry St. 1960 1970 AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val> for the first for the first tage [SPLIT] for the first for 2000 TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr> $_f_f_f_f_f$ TAG [SPLIT] $_f_f_f_f_f$ 2030 CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>

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6/38 Fig.2A. 10 20 30 ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala> _a_TRYPSIN SIGNAL SEQUENCE___a_ 50 60 90 80 AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC Arg Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly> 100 110 120 130 ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC Ile Tyr Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala> _b__b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__b_ 140 160 170 180 TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln> _b__b__b__ang2 fibrinogen-like domain #1__b_ 190 200 210 CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu> _b__b__b_ANG2 FIBRINOGEN-LIKE DOMAIN #1___b_ 230 240 250 270 TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly> _b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1_ 280 290 300 **★**× ---AAT GAG TIT GIT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu> _b__b__b__ang2 fibrinogen-like domain #1__b__b_ 340 350 . * go, *part, * * opt | . * . AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu> _b__b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__ 370, 380 380 390 St. Sat TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile> _b_ANG2 FIBRINOGEN-LIKE DOMAIN #1__b_b_b_-> 450 -CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile> _b__b__b__ANG2 FIBRINOGEN-LIKE DOMAIN #1__b__b_

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Fig.2C 900 860 880 890 GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly> ___d__d__d_ANG2 FIBRINOGEN-LIKE DOMAIN#2___d__d__d__d__ 930 920 TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val> 950 960 970 980 TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu> _d__ang2 fibrinogen-like domain#2___d__d 1000 1030 1010 AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe> 1070 **★** TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly> __d__d__d_ANG2 FIBRINOGEN-LIKE DOMAIN#2___d__d__d__> 1100 1110 1120 1090 * CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC CAA CCA GGA Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly> 1170 1150 1160 1130 AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys Ile Cys> d__d_ d__ANG2 FIBRINOGEN-LIKE DOMAIN#2___d_d__d__d_ 200 1210 1200 AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA TGT Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys> _d__d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d__d__d___> 7 1 1 442 STORY STORY 1240 GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn> ___d__

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TAT Tyr	AAA Lys	Met b	Gly	TTT Phe	Gly	Asn	CCC	* TCC Ser	Gly	GAA Glu	TAT Tyr	Trp b	Leu b	GGG Gly>
TAT Tyr	AAA Lys	Met b	Gly b	TTT Phe	Gly	Asn	CCC	* TCC Ser	Gly	GAA Glu	TAT Tyr	Trp b	Leu	GGG Gly>
TAT Tyr	AAA Lys b	Met b 28 TTT	Gly b 30 * ATT	TTT Phe ANG1	Gly FIB	Asn RINO 290 * ATT	CCC Pro GEN-1	TCC Ser LIKE	Gly DOM 300 *	GAA Glu AIN_	TAT Tyr b	Trp b3	Leu b 10 * ATG	GGG Gly>
TAT Tyr AAT Asn	AAA Lys b GAG Glu	Met b28 TTT Phe	Gly b 30 * ATT Ile	TTT Phe ANG1 TTT Phe	Gly FIB GCC Ala	Asn RINO 290 * ATT Ile	CCC Pro GEN-	* TCC Ser LIKE AGT Ser	Gly DOM * CAG Gln	GAA Glu AIN_ AGG Arg	TAT TYP CAG	Trp 3 TAC Tyr	Leu b 10 * ATG Met	GGG Gly> CTA Leu>
TAT Tyr AAT Asn	AAA Lys b GAG Glu	Met b28 TTT Phe	Gly b 30 * ATT Ile	TTT Phe ANG1 TTT Phe	Gly FIB GCC Ala	Asn RINO 290 * ATT Ile	CCC Pro GEN-	* TCC Ser LIKE AGT Ser	Gly DOM * CAG Gln	GAA Glu AIN_ AGG Arg	TAT TYP CAG	Trp 3 TAC Tyr	Leu b 10 * ATG Met	GGG Gly>
TAT Tyr AAT Asn	AAA Lys b GAG Glu b	Met b28 TTT Phe	Gly b 30 * ATT Ile	TTT Phe ANG1 TTT Phe ANG1	Gly FIB GCC Ala	Asn RINO 290 * ATT Ile	CCC Pro GEN- ACC Thr GEN-	* TCC Ser LIKE AGT Ser	Gly DOM * CAG Gln	GAA Glu AIN_ AGG Arg AIN_	TAT Tyr b CAG Gln b	Trp 3 TAC Tyr	Leu b 10 * ATG Met	GGG Gly> CTA Leu>
TAT Tyr AAT Asn	AAA Lys b GAG Glu b	Met b 28 TTT Phe b	Gly b 30 * ATT Ile	TTT Phe ANG1 TTT Phe ANG1	Gly FIB GCC Ala	Asn RINO 290 * ATT Ile	CCC Pro GEN- ACC Thr GEN-	TCC Ser LIKE AGT Ser LIKE	Gly DOM * CAG Gln	GAA Glu AIN_ AGG Arg AIN_	TAT TYP CAG	Trp 3 TAC Tyr	Leu b 10 * ATG Met	GGG Gly> CTA Leu>
TAT Tyr AAT Asn	AAA Lys b GAG Glu b 320	Met b	Gly b 30 ATT Ile b TTA	TTT Phe ANG1 Phe ANG1 ANG1 ANG1 ANG1	GCC Ala FIB	Asn RINO 290 * ATT Ile RINO *	CCC Pro GEN- ACC Thr GEN-1	TCC Ser LIKE AGT Ser LIKE	Gly DOM. 300 CAG Gln DOM.	GAA Glu AIN_ AGG Arg AIN_	TAT Tyr b CAG Gln b 350	TAC TAC TYT TAT	Leu b 10 * ATG Met b * TCA	GGG Gly> CTA Leu> CTA CAG
TAT Tyr AAT Asn AGA Arg	AAA Lys b GAG Glu b 320 ATT Ile	Met b	Gly b 30 ATT Ile b TTA Leu	TTT Phe ANG1 TTT Phe ANG1 330 ATG	GCC Ala FIB	Asn RINO 290 * ATT Ile RINO TGG Trp	CCC Pro GEN-1 ACC Thr GEN-1 34 GAA Glu	TCC Ser LIKE AGT Ser LIKE 40 *	Gly DOM CAG Gln DOM AAC Asn	GAA Glu AIN_ AGG Arg AIN_ CGA	TAT TYT b CAG GIn b GCC Ala	TAC TYC TAT TYT	Leu b 10 * ATG Met b TCA Ser	GGG Gly> CTA Leu> CTA CAG Gln>
TAT Tyr AAT Asn AGA Arg	AAA Lys b GAG Glu b 320 ATT Ile	Met b	Gly b 30 ATT Ile b TTA Leu	TTT Phe ANG1 TTT Phe ANG1 330 ATG	GCC Ala FIB	Asn RINO 290 * ATT Ile RINO TGG Trp	CCC Pro GEN-1 ACC Thr GEN-1 34 GAA Glu	TCC Ser LIKE AGT Ser LIKE 40 *	Gly DOM CAG Gln DOM AAC Asn	GAA Glu AIN_ AGG Arg AIN_ CGA	TAT TYT b CAG GIn b GCC Ala	TAC TYC TAT TYT	Leu b 10 * ATG Met b TCA Ser	GGG Gly> CTA Leu> CTA Ceu> CAG Gln>
TAT Tyr AAT Asn AGA Arg	AAA Lys b GAG Glu b 320 ATT Ile	Met b	Gly b 30 ATT Ile b TTA Leu	TTT Phe ANG1 TTT Phe ANG1 330 ATG	GCC Ala FIB	Asn RINO 290 * ATT Ile RINO TGG Trp	CCC Pro GEN-1 ACC Thr GEN-1 34 GAA Glu	TCC Ser LIKE AGT Ser LIKE 40 *	Gly DOM CAG Gln DOM AAC Asn	GAA Glu AIN_ AGG Arg AIN_ CGA	TAT TYT b CAG GIn b GCC Ala	TAC TYC TAT TYT	Leu b 10 * ATG Met b TCA Ser	GGG Gly> CTA Leu> CTA Ceu> CAG Gln>
TAT Tyr AAT Asn AGA Arg	AAA Lys b GAG Glu b 320 ATT Ile	Met b	Gly b 30 ATT Ile b TTA Leu	TTT Phe ANG1 TTT Phe ANG1 330 ATG	GCC Ala FIB	Asn RINO 290 * ATT Ile RINO TGG Trp	CCC Pro GEN-1 ACC Thr GEN-1 34 GAA Glu	TCC Ser LIKE AGT Ser LIKE 40 *	Gly DOM CAG Gln DOM AAC Asn	GAA Glu AIN_ AGG Arg AIN_ CGA	TAT TYT b CAG GIn b GCC Ala	TAC TYC TAT TYT	Leu b 10 * ATG Met b TCA Ser	GGG Gly> CTA Leu> CTA CAG
TAT Tyr AAT Asn AGA Arg	GAG Glu b ATT Ile b GAC	Met b	Gly b ATT Ile b TTA Leu b TTC	TTT Phe ANG1 TTT Phe ANG1 330 ATG Met ANG1	GCC Ala FIB	ASM RINO 290 * ATT Ile RINO TGG Tip RINO 380 *	ACC Thr GAA Glu GEN-	TCC Ser LIKE AGT Ser LIKE GGG Gly LIKE	Gly DOM 300 CAG Gln DOM AAC Asn DOM	GAA Glu AIN_ AGG Arg AIN_ CGA Arg AIN_	TAT TYT b CAG GIn b GCC Ala b	TAC TYT TAT TYT TAT TYT	Leu b 10 * ATG Met b * TCA Ser b 00 * AGG	GGG Gly> CTA Leu> CAG Gln> CAG TTG
TAT Tyr AAT Asn AGA Arg TAT Tyr	GAG Glu b 11e b GAC Asp	Met b	Gly b ATT Ile b TTA Leu 70 TTC Phe	TTT Phe ANG1 TTT Phe ANG1 330 ATG Met ANG1 CAC His	GCC Ala FIB	ASM RINO 290 * ATT Ile RINO * TGG Trp RINO 380 * GGA Gly	CCC Pro GEN-1 ACC Thr GAA GLU GEN-1 AAT Asn	TCC Ser LIKE AGT Ser LIKE GGG Gly LIKE	Gly DOM CAG Gln DOM AAC Asn DOM 390 AAG Lys	GAA Glu AIN_ AGG Arg AIN_ CGA Arg AIN_ CAA Gln	TAT TYT b CAG GIn b GCC Ala b	TAC TYT TAT TYT TAT TYT	Leu b 10 * ATG Met b * TCA Ser b 00 * AGG Arg	GGG Gly> CTA Leu> CAG Gln>

Fig.3	B. '	410			420			43	0		4	140			450
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	Asn	Cys	Met	Cys	Lys	Cys	Ala	Leu	Met	Leu	Thr	Gly	Gly	Trp	Trp>
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Fig.3	C.	•	8	20	•		830	, ·	•	840		•	8	50	•
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•	1	040		•	1050	٠		10	60	•	1	070		•	1080
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	Leu	Thr	AAG Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe>
		d	d	d	d	d	dF	C TA	G	.d	d	_d	d		d>
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	TAT	CCC	AGC. Ser	GAC Asp	ATC Ile	GCC Ala	GTG Val	GAG Glu	TGG	GAG Glu	AGC Ser	Asn	Gly	Gln	CCG Pro> d>
	1	220		, ;	1230			12	40	195 •	. 1	250	K 75 - 11	ng•	1260
	GAG Glu	AAC Asn	AAC Asn	TAC.	AAG Lys	ACC Thr	ACG Thr	CCT Pro	Pro	GTG Val	CTG	GAC Asp	TCC Ser	GAC Asp	GGC Gly> d>
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Fig.3D.

	*	12	70 *	•	1	280			1290		_	130	00	
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CAG	CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT	CTG
GIn	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu>
	d	d(d(d	d	dF	C TA	.G	d	d	d	d	a	d>
	*	136	•		1.	370			1380			139	90	
CAC	AAC	CAC	TAC	ACC.	CXC	880	100	-	TO C	000	·mom		*	AAA
His	Asn	His	TVT	Thr	Gln	TAKE	Cor	Lou	200	CIG	TCT	CCG	GGT	AAA Lys>
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Tyr	Gln	Ala	Gly	Phe	Asn	Lvs	Ser	Glv	Ile	TVÝ	Thr	Tle	ብጥት ፕሂተ	Ile>
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Asn	Gly .	Gly	Gly	Trp	Thr	Val-	Ile	Gln	His	Arg	Glu	Asp	Gly	Ser>
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Fig.3E.

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ATT	ACC	AGT	CAG	AGG	CAG	TAC	ATG	CTA	AGA	ATT	GAG	TTA	ATG	GAC
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Trp	Glu	Gly	Asn	Arg	Ala	Tyr	Ser	Gln	Tyr	Asp	Arg	Phe	His	Ile>
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GIY	ASN	GIU E	Lys e	GIN	Asn	Tyr	Arg	Leu	Tyr	Leu	Lys	Gly	His	Thr>
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TTC	AGC	ACT	AAA	GAT	GCT	GAT	AAT	GAC	AAC	TGT	ATG	TGC	AAA	TGT
Phe	Ser	Thr	Lys	Asp	Ala	Asp	Asn	Asp	Asn	Cys	Met	Cvs	Lvs	Cvs>
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	Leu	Met	Leu	Thr	Gly	Gly	Trp	Trp	Phe	.Asp	Ala	Cys	Gly	Pro>
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AGA	GAC	TGT	GCT	GAA	GTA	TTC	AAA	TCA	GGA	CAC	ACC	ACA	AAT	GGC
Arg	Asp	Cys	Ala	Glu	Val	Phe	Lys	Ser	Gly	His	Thr	Thr	Asn	Gly>
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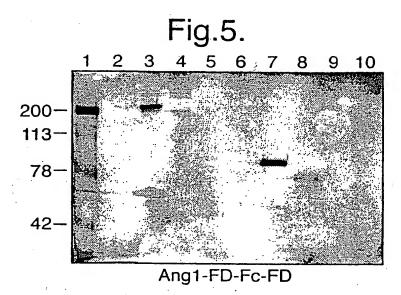
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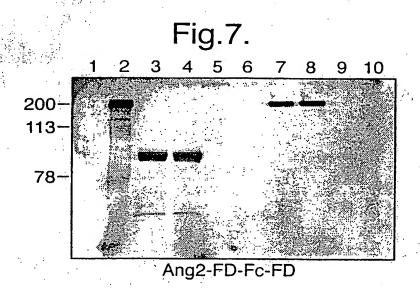
Fig.	4C		8	20	•		830		*	840		•	8	50	•
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	Ala	Leu	Pro	Ala dc	Pro	Ile do	Glu	Lys	Thr 	Ile	Ser	Lys	Ala	Lys d	Gly>
	Ala ——C	Leu Leu CCC	Pro 1° 109 CGA	Ala 10 0 * GAA	Pro	Ile do 11 CAG	Glu if(.00 GTG	Lys C TAC	Thr Jo t ACC	Ile dc .110 *	Ser	Lys Lys CCA	Ala dc 112 TCC	Lys dc cgg	Gly>
	Ala ——C	Leu Leu CCC	Pro 1° 109 CGA	Ala 10 0 * GAA	Pro	Ile d 11 CAG Gln	Glu iF(.00 GTG Val	Lys C TAC	Thr J * ACC Thr	Ile	Ser	Lys Lys CCA Pro	Ala dc 112 TCC Ser	Lys do cgg Arg	Gly> i>
	CAG Gln	Leu Leu CCC	Pro 1° 109 CGA	Ala dc o GAA Glu	Pro CCA Pro	Ile d 11 CAG Gln	Glu iF(.00 GTG Val	Lys TAC TAC	Thr ACC Thr	Ile	Ser	Lys Lys CCA Pro	Ala dc 112 TCC Ser	Lys cgg Arg	Gly> d> GAT Asp>
	CAG Gln	CCC Pro	Pro 109 CGA Arg	Ala dc 0 GAA Glu dc 1	CCA Pro	Ile d 11 CAG Gln,	Glu iFC	TAC TY TAC TY TAC	Thr ACC Thr 60	Ile	CCC Pro	CCA Pro	Ala dc 112 TCC Ser	Lys dc c CGG Arg lc	Gly> d> GAT Asp> d>
	CAG Gln ——G GAG G1u	CCC Pro	Pro 109 CGA Arg ACC Thr	Ala O GAA Glu AAG Lys	CCA Pro 140 AAC ASD	Ile Ile CAG Gln CAG CAG Gln	Glu if(TAC TYP TAC TYP TAC TAC TST AGC Ser	Thr ACC Thr CTG Leu	Ile illo t CTG Leu l ACC Thr	CCC Pro	Lys CCA Pro CTG Leu	Ala i 112 TCC Ser i GTC Val	Lys CGG Arg Lys AAA Lys	Gly> d> GAT Asp> d> 170 * GGC Gly>
	CAG Gln ——G GAG G1u	CCC Pro	Pro 109 CGA Arg ACC Thr	Ala ic GAA Glu ic AAG Lys	CCA Pro 140 AAC ASD	CAG CAG CAG CIn	Glu if(TAC TYP TAC TYP TAC TAC TST AGC Ser	ACC Thr CTG Leu	Ile illo t CTG Leu l ACC Thr	CCC Pro	Lys CCA Pro CTG Leu	Ala i 112 TCC Ser i GTC Val	Lys CGG Arg Lys AAA Lys	Gly>d> GAT Asp>d> 170 * GGC
	CAG Gln ——G GAG Glu ——G	CCC Pro 30 CTG Leu	Pro 109 CGA Arg ACC Thr	Ala d GAA Glu AAG Lys d 0	CCA Pro 140 AAC Asn	CAG CAG CAG CIn L CAG CIN CAG CIN CAG CIN CAG	GTC Val	TAC TYT TAC TYT TAC TAC TAC TAC	Thr ACC Thr CTG Leu	CTG Leu ACC Thr	CCC Pro 11 TGC Cys	CCA Pro CTG Leu	Ala ic 112 TCC Ser ic GTC Val	Lys CGG Arg Lys AAA Lys O	Gly> d> GAT Asp> d> 170 * GGC Gly> d>
	CAG Gln GAG Glu TTC Phe	CCC Pro 30 CTG Leu TAT Tyr	Pro 109 CGA Arg ACC Thr 118 CCC Pro	Ala O GAA Glu AAG Lys O AGC Ser	CCA Pro 140 AAC ASD GAC ASD	CAG Gln CAG Gl	GTC Val GTC Val GTC Val GTC At	TAC TYT TAC TYT TAC TAC TAC STAC GTG Val	Thr ACC Thr CTG Leu GAG Glu	CTG Leu ACC Thr 200 TGG Trp	CCC Pro 11 TGC Cys GAG G1ü	CCA Pro 60 CTG Leu	Ala dc 112 TCC Ser dc GTC Val dc 121 AAT ASD	Lys CGG Arg Lys AAA Lys GGG Gly	Gly> d> GAT Asp> d> 170 GGC Gly> d> CAG Gln>
	CAG Gln GAG Glu TTC Phe	CCC Pro 30 CTG Leu TAT Tyr	Pro 109 CGA Arg ACC Thr 118 CCC Pro	Ala dC f GAA Glu Lys Lys AGC Ser	CCA Pro 140 AAC ASD GAC ASD	CAG Gln CAG Gl	GTC Val GTC Val GTC Val GTC At	TAC TYT TAC TYT TAC TAC TAC STAC GTG Val	Thr ACC Thr CTG Leu GAG Glu	CTG Leu ACC Thr 200 TGG Trp	CCC Pro 11 TGC Cys GAG Glü	CCA Pro 60 CTG Leu	Ala dc 112 TCC Ser dc GTC Val dc 121 AAT ASD	Lys CGG Arg AAA Lys GGG Gly	Gly>d> GAT Asp>d> 170 GGC Gly>d> CAG
	CAG Gln GAG Glu TTC Phe 12	CCC Pro 30 CTG Leu TAT Tyr 20	Pro 109 CGA Arg ACC Thr 118 CCC Pro	Ala ic GAA Glu ic AAG Lys ic O * AGC Ser ic	CCA Pro 140 AAC ASN GAC ASP	CAG Gln CAG Gl	GTC Val GTC Val GTC Val GTC At	TAC TYT TAC TYS TAC TYS TAC	Thr ACC Thr CTG Leu GAG Glu	Ile 110 CTG Leu ACC Thr 200 TGG Trp	CCC Pro 11 TGC Cys GAG G1ü	CCA Pro 60 CTG Leu	Ala ic 112 TCC Ser ic GTC Val ic 121 AAT ASD	Lys CGG Arg AAA Lys GGG Gly	Gly> d
	CAG Gln GAG Glu TTC Phe 12 CCG Pro	CCC Pro 30 CTG Leu TAT Tyr GAG Glu	Pro 109 CGA Arg ACC Thr 118 CCC Pro AAC AAC	Ala d O O SAA Glu AAG Lys C O AGC Ser AAC AAC	CCA Pro 140 AAC ASD GAC ASP TAC Tyr	CAG Gln CAG Gln ATC Ile	GTC Val GTC Val GTC Ala ACC Thr	TAC TYT TAC TYT TAC TYC TAC TAC TAC	ACC Thr CTG Leu GAG Glu CCT Pro	ACC Thr CCC Pro	CCC Pro 11 TGC Cys GAG G1u 12 GTG Val	CCA Pro 60 CTG Leu AGC Ser CTG Leu	Ala illo illo GTC Ser Illo GTC Val Illo 121 AAT AST AST GAC ASP	CGG Arg AAA Lys GGG Gly TCC Ser	Gly> d

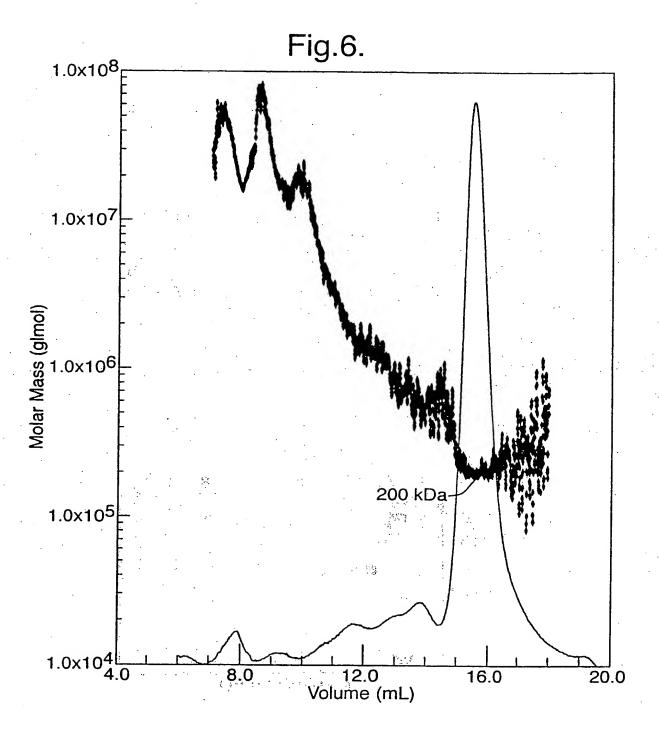
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	Phe	Lys	TCA Ser	* GGA Gly	His	ACC Thr	* ACA Thr	Asn	GGC Gly	ATC Ile	TAC Tyr	Thr	TTA Leu	* ACA Thr	Phe>
	Phe	Lys	TCA Ser	* GGA Gly	His	ACC Thr	* ACA Thr	Asn	GGC Gly	ATC Ile	TAC Tyr	Thr	TTA Leu	* ACA Thr	
	Phef	Lys	TCA Ser	GGA Gly	His	ACC Thr	* ACA Thr	Asn	GGC Gly LIKE	ATC Ile	TAC Tyr AIN_	Thr	TTA Leu	* ACA Thr	Phe>
	Phef	Lys	TCA Ser	GGA Gly	His ANG2	ACC Thr	* ACA Thr	Asn GEN-I	GGC Gly LIKE	ATC Ile	TAC Tyr AIN_	Thr E	TTA Leu	* ACA Thr	Phe> f>
	Phef	Lys 90 *	TCA Ser E	GGA Gly E	His ANG2 1500	ACC Thr FIBI	ACA Thr RINO	Asn GEN-1 15]	GCC Gly GGC	ATC Ile DOM	TAC Tyr AIN_ 1!	Thr f 520 GAC	TTA Leu f	* ACA Thr E * GAA	Phe> f> 1530 * GCT
	Phe f 14 CCT Pro	Lys 90 * AAT Asn	TCA Ser E	GGA Gly E ACA	His ANG2 1500 GAA Glu	ACC Thr FIBI GAG Glu	ACA Thr RINOC	Asn GEN-1 151 AAG Lys	GGC Gly LIKE	ATC Ile DOM	TAC Tyr AIN_ 1! TGT Cys	Thr f 520 GAC Asp	TTA Leu f	* ACA Thr f GAA Glu	Phe> f> 1530 GCT Ala>
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	Phe f 14 CCT Pro	Lys 90 * AAT Asn	TCA Ser E	GGA Gly E ACA	His ANG2 1500 GAA Glu	ACC Thr FIBI GAG Glu FIBI	ACA Thr RINO	Asn GEN-1 151 AAG Lys	GGC Gly LIKE GCC Ala	ATC Ile DOM	TAC Tyr AIN_ 1! TGT Cys	Thr f 520 GAC Asp	TTA Leu f ATG Met f	* ACA Thr f GAA Glu f	Phe> f> 1530 GCT Ala>
	Phe f 14 CCT Pro	Lys 90 * AAT Asn	TCA Ser E	GGA Gly E ACA	His ANG2 1500 GAA Glu	ACC Thr FIBI GAG Glu FIBI	ACA Thr RINOC	Asn GEN-1 151 AAG Lys	GGC Gly LIKE GCC Ala	ATC Ile DOM	TAC Tyr AIN_ 1! TGT Cys	Thr f 520 GAC Asp	TTA Leu f	* ACA Thr f GAA Glu f	Phe> f> 1530 GCT Ala>
	Phef	PAT ASN	TCA Ser TCT Ser 154	GGA Gly ACA Thr	His ANG2 1500 GAA Glu ANG2	ACC Thr FIBI GAG Glu FIBI	ACA Thr RINOC ATC Ile RINOC 550	Asn CEN-1 151 AAG Lys GEN-1	GGC Gly LIKE LO GCC Ala LIKE	ATC Ile DOM	TAC Tyr AIN_ 1! TGT Cys AIN_	Thr f 520 GAC Asp f	TTA Leu f ATG Met f 157	ACA Thr GAA Glu f	Phe> f> 1530 GCT Ala>
	Phef 14 CCT Prof GGA Gly	90 AAT ASN GGA GIY	TCA Ser TCT Ser GGC GJY	ACA Thr GGG GGG	His ANG2 1500 GAA Glu ANG2 TGG	ACC Thr FIBI GAG Glu FIBI 15	ACA Thr RINO ATC Tle RINO 550 ATT Ile	ASN GEN-I 151 AAG Lys GEN-I ATT Ile	GGC Gly LIKE GCC Ala LIKE CAG Gln	ATC Ile DOM TAC Tyr DOM CGA Arg	TAC TYF AIN 1! TGT CYS AIN CGT Arg	Thr f GAC Asp GAG GAG GAG Glu	TTA Leu f ATG Met f 157	ACA Thr GAA Glu GU GGC GGC Gly	Phe> f> 1530 GCT Ala> f> AGC Ser>
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	Phe f 14 CCT Pro f GGA Gly f	90 AAT ASn GGA GU	TCA Ser TCT Ser GGC GJY	GGA Gly ACA Thr GGG Gly	His ANG2 1500 GAA Glu ANG2 TGG Trp ANG2	ACC Thr FIBI GAG Glu FIBI 15	ACA Thr RINO ATC Tle RINO 550 ATT Ile	ASN GEN-1 151 AAG Lys GEN-1 ATT Ile GEN-1	GGC Gly LIKE CCC Ala LIKE CAG Gln LIKE	ATC Ile DOM TAC Tyr DOM CGA Arg	TAC TYT AIN 1! TGT CYS AIN CGT AIG AIN	Thr f 520 GAC Asp f GAG Glu f	TTA Leu f ATG Met f 157	ACA Thr GAA Glu GGC Gly f	Phe> f> 1530 * GCT Ala> f> AGC Ser> f>
	Phe f 14 CCT Pro f GGA Gly f	90 AAT ASN GGA GIY	TCA Ser TCT Ser GGC GJY	GGA Gly ACA Thr GGG Gly	His ANG2 1500 GAA Glu ANG2 TGG	ACC Thr FIBI GAG Glu FIBI 15	ACA Thr RINO ATC Tle RINO 550 ATT Ile	ASN GEN-I 151 AAG Lys GEN-I ATT Ile	GGC Gly LIKE CCC Ala LIKE CAG Gln LIKE	ATC Ile DOM TAC Tyr DOM CGA Arg	TAC TYT AIN 1! TGT CYS AIN CGT AIG AIN	Thr f GAC Asp GAG GAG GAG Glu	TTA Leu f ATG Met f 157	ACA Thr GAA Glu GGC Gly f	Phe> f> 1530 GCT Ala> f> AGC Ser>
	Phe for the second seco	AAT ASN GGA Gly	TCA Ser TCT Ser GGC Gly	GGA Gly ACA Thr GGG Gly	His ANG2 1500 GAA Glu ANG2 TGG Trp ANG2	ACC Thr FIBI GAG Glu FIBI ACA Thr FIBI	ACA Thr RINO ATC Ile RINO 550 ATT Ile RINO	ASN GEN-1 151 AAG Lys GEN-1 ATT Ile GEN-1	GGC GIY LIKE GCC Ala LIKE CAG GIn LIKE	ATC Ile DOM TAC Tyr DOM CGA Arg DOM	TAC TYT AIN_ 1! TGT CYS AIN_ CGT AIG AIN_	GAC ASP GAG Glu f	TTA Leu f ATG Met f 157 GAT Asp f	ACA Thr GAA Glu GGC Gly f	Phe> f > 1530 * GCT Ala> f > AGC Ser> f >
	Phe f 14 CCT Pro f GGA Gly 15 GTT Val	90 AAT ASN GGA Gly B0 GAT ASP	TCA Ser TCT Ser GGC Gly TTT Phe	GGA Gly ACA Thr GGG Gly CAG Gln	His ANG2 1500 GAA Glu ANG2 TGG Trp ANG2 L590 AGG Arg	ACC Thr FIBI GAG Glu FIBI ACA Thr FIBI	ACA Thr RINO ATC Tile RINO ATT Ile RINO TGG Trp	ASN GEN-I 151 AAG Lys GEN-I 11e GEN-I 160 AAA	GGC GIY GCC Ala LIKE CAG GIn LIKE	TAC TYP DOM CGA ATG DOM TAT TYP	TAC TYT AIN_ 1: TGT CYS AIN_ AIN_ AAA LYS	GAG	TTA Leu f ATG Met f 157 GAT ASP f GGA Gly	ACA Thr f GAA Glu f GGC Gly f TTT Phe	Phe> f> f> 1530 * GCT Ala> f> AGC Ser> f> 1620 * GGT Gly>
	Phe f 14 CCT Pro f GGA Gly 15 GTT Val	90 AAT ASN GGA Gly B0 GAT ASP	TCA Ser TCT Ser GGC Gly TTT Phe	GGA Gly ACA Thr GGG Gly CAG Gln	His ANG2 1500 GAA Glu ANG2 TGG Trp ANG2 L590 AGG Arg	ACC Thr FIBI GAG Glu FIBI ACA Thr FIBI	ACA Thr RINO ATC Tile RINO ATT Ile RINO TGG Trp	ASN GEN-I 151 AAG Lys GEN-I 11e GEN-I 160 AAA	GGC GIY GCC Ala LIKE CAG GIn LIKE	TAC TYP DOM CGA ATG DOM TAT TYP	TAC TYT AIN_ 1: TGT CYS AIN_ AIN_ AAA LYS	GAG	TTA Leu f ATG Met f 157 GAT ASP f GGA Gly	ACA Thr f GAA Glu f GGC Gly f TTT Phe	Phe> f > 1530 * GCT Ala> f > AGC Ser> f > 1620 * GGT
	Phe f 14 CCT Pro f GGA Gly 15 GTT Val	90 AAT ASN GGA Gly B0 GAT ASP	TCA Ser TCT Ser GGC Gly TTT Phe	GGA Gly ACA Thr GGG Gly CAG Gln	His ANG2 1500 GAA Glu ANG2 TGG Trp ANG2 L590 AGG Arg	ACC Thr FIBI GAG Glu FIBI ACA Thr FIBI	ACA Thr RINO ATC Ile RINO ATT Ile RINO TGG Trp RINO	ASN GEN-I 151 AAG Lys GEN-I 160 AAA Lys GEN-I	GGC Gly LIKE GCC Ala LIKE GAG Gln GIKE	TAC TYP DOM CGA Arg DOM TAT TYP TOM	TAC TYT AIN_ 1: TGT CYS AIN_ AIN_ AAA LYS	GAG	TTA Leu f ATG Met f GAT Asp f GGA Gly f	* ACA Thr GAA Glu	Phe> f> f> 1530 * GCT Ala> f> AGC Ser> f> 1620 * GGT Gly>
	Phe f 14 CCT Pro f GGA Gly f 15 GTT Val f	90 AAT Asn GGA Gly GAT Asp	TCA Ser TCT Ser GGC Gly TTT Phe	ACA Thr GGG Gly CAG Gln	His ANG2 1500 GAA Glu ANG2 Trp ANG2 L590 AGG Arg	ACC Thr FIBI	ACA Thr RINO ATC Ile RINO 550 ATT Ile RINO TGG Trp RINO 40	ASN GEN-I 151 AAG LYS GEN-I 11e GEN-I 160 AAA LYS GEN-I	GGC Gly LIKE CAG GIn LIKE GAA GIU LIKE	TAC TYP DOME TAT TYP DOME 650	TAC TYP AIN_ 1: TGT CYS AIN_ CGT AIG AIN_ 1: AAA LYS AIN_	GAC ASP GAG Glu f GTG Val f	TTA Leu f ATG Met f 157 GAT Asp f GGA Gly f	* ACA Thr GAA Glu f GGC Gly TTT Phe f	Phe> f> f> 1530 GCT Ala> f> AGC Ser> f> 1620 GGT Gly> f>
	Phe f 14 CCT Pro f GGA Gly f 15 GTT Val f	90 AAT Asn GGA Gly GAT Asp	TCA Ser TCT Ser GGC Gly TTT Phe	ACA Thr GGG Gly CAG Gln	His ANG2 1500 GAA Glu ANG2 Trp ANG2 L590 AGG Arg	ACC Thr FIBI	ACA Thr RINO ATC Ile RINO 550 ATT Ile RINO TGG Trp RINO 40	ASN GEN-I 151 AAG LYS GEN-I 11e GEN-I 160 AAA LYS GEN-I	GGC Gly LIKE CAG GIn LIKE GAA GIU LIKE	TAC TYP DOME TAT TYP DOME 650	TAC TYP AIN_ 1: TGT CYS AIN_ CGT AIG AIN_ 1: AAA LYS AIN_	GAC ASP GAG Glu f GTG Val f	TTA Leu f ATG Met f 157 GAT Asp f GGA Gly f	* ACA Thr GAA Glu f GGC Gly TTT Phe f	Phe> f> f> 1530 GCT Ala> f> AGC Ser> f> 1620 GGT Gly> f>
	Phe f 14 CCT Pro f GGA Gly f 15 GTT Val f	AAT ASN GGA Gly GAT ASP	TCA Ser TCT Ser TTT 154 TTT Phe 163	* GGA GIY ACA Thr CGG GIY CAG GIN	His ANG2 1500 GAA Glu ANG2 TGG Trp ANG2 L590 AGG Arg	ACC Thr FIBI GAG Glu FIBI ACA Thr FIBI ACT Thr FIBI	ACA Thr RINO ATC Ile RINO 550 ATT Ile RINO TGG Trp RINO TGG	ASN GEN-I 151 AAG Lys GEN-I 11e GEN-I 160 AAA Lys GEN-I	GGC Gly LIKE GCC Ala LIKE GAA Glu LIKE	TAC TYP DOM CGA Arg DOM TAT TYP DOM AAT	TAC TYT AIN_ 1! TGT CYS AIN_ AIN_ AAA LYS AIN_ GAG	GAC ASP GAG Glu f GTG Val	TTA Leu f ATG Met f GAT Asp GGA Gly f 166	* ACA Thr f GAA Glu f GGC Gly f TTT Phe f TCG	Phe> f> f> 1530 * GCT Ala> f> AGC Ser> f> 1620 * GGT Gly>

Fig.4E.

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LIG	WCT	AAT	CAG	CAA	CGC	TAT	GTG	CTT	AAA	ATA	CAC	CTT	AAA	GAC
Leu	e mr	e ASN	GIN	GIN	Arg	Tyr	Val	Leu	Lys	Ile	His	Leu	Lys	<qaa< td=""></qaa<>
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														Leu>
	£	£	f	ANG2	FIB	RINO	GEN-I	LIKE	DOM	AIN	F	f :	F :	£>
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	*		*	*)	* 1		*	*		*		*	*
TCA	AGT	GAA	GAA	CTC	AAT	TAT	AGG	ATT	CAC	CTT	AAA	GGA	CTT	ACA
Ser	Ser	Glu	Glu	Leu	Asn	Tyr	Arg	Ile	His	Leu	Lys	Gly	Leu	Thr>
<u> </u>	£	£	£	ANG2	FIB	RINO	GEN-1	LIKE	DOM	AIN_	E	£:	E:	£>
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Glv	Thr	Ala	Gly	Tare	TIA	AGC	AGC Co~	TIO	AGC	CAA	CCA,	GGA	AAT	GAT Asp>
	f	f.	f F	מעם מאמ	TIE	SEL	26Y-1	TTVE	DOM	GIII ATNI 4	t PIO	eGΣΥ	ASN c	жр> >
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ŤTT	AGC	ACA	AAG	GAT	GGA	GAC	AAC	GAC	AAA	TGT	ATT	TGC	AAA	TGT
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	£	£	f	ANG2	FIB	RINO	ĠEN-I	LIKE	DOM	AIN_	Ė	£	£	f>
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Ser	e GIII													Pro>
	L		I.——'	ANGZ	FIBI	CINOC	≥EM-1	PIKE	DOM	7TN"	c	E	F	£>
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TCG	CTC	AAG	GCĆ	ACA	ACC	ATG	ATG	ATC	CGA	CCA	GCA	GAT	TTC	TGA
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									MAIN					
	7.		' -					;						







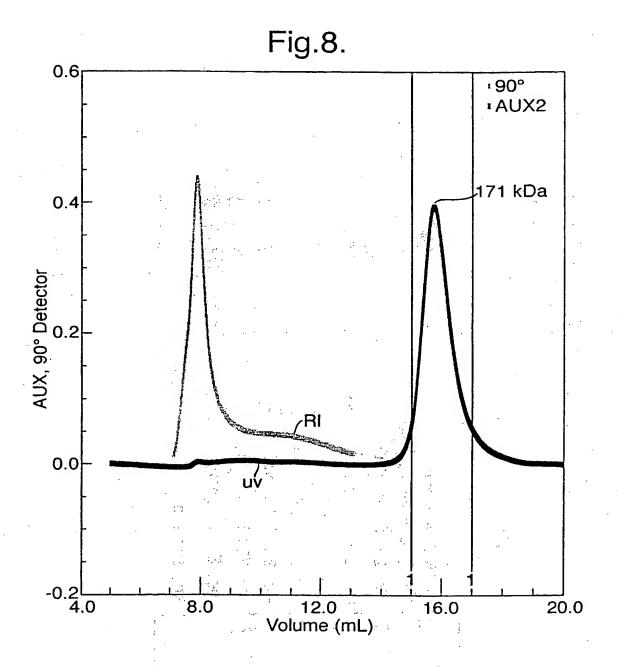


Fig.9.

Ang1*(0,1µg/ml) 2.5Ru Ang1-Bowtie (0.1µg/ml) 1*-(0.2µg/ml)(50Ru) 1-B.T.(0.2µg/ml) 1*-(0.4µg/ml) 100Ru 1-B.T.(0.4µg/ml) 1*-(0.8µg/ml)(200Ru) 1-B.T.(0.8µg/ml)

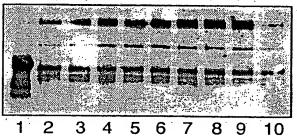
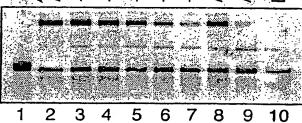
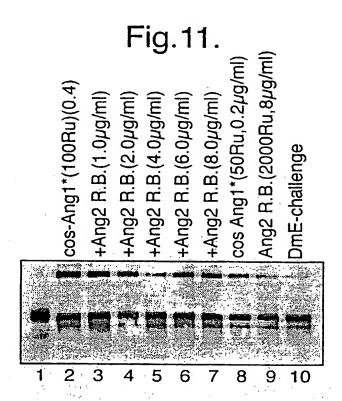


Fig. 10.

Ang1*(100Ru)(0.4µg/m +Ang2 B.T.(1.0µg/ml) +Ang2 B.T.(2.0µg/ml) +Ang2 B.T.(4.0µg/ml) +Ang2 B.T.(6.0µg/ml) +Ang2 B.T.(8.0µg/ml) Ang1*(50Ru)(0.2µg/ml) Ang2 B.T.(8.0µg/ml) DmE-challenge

5.7 NMolar monomer





Ang2-Bowtie(8µg/ml) Ang1-wT(50Ru)(0.2µg/ml) Ang1-wT(50Ru)(0.2µg/ml) Ang1-wT(50Ru)(0.2µg/ml) (0.2:2.0) (0.2:4.0) (0.2:6.0) (0.2:8.0) Ang1-wT(25Ru)(0.1µg/ml) OmE-challenge

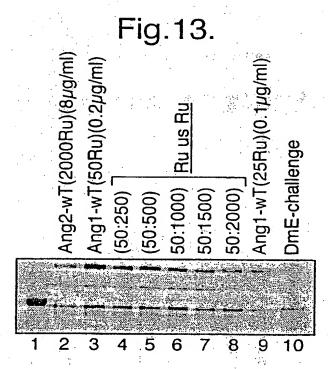


Fig.14A.

		D	• • •	••										
			10			20			30			4	40	
ATG	GCI	. cee	- -	- GGG	CAC	CCT.	TCC.	с тс	*		*		•	•
Met	Ala	Arg	Pro	Glv	Gln	Ara	Trn	Leu	CIV	TAG	TGG	CTT	GTG	GCG Ala>
	_a	a_EL	K-L	ECTO	DOMA	IN 1	(WI	TH S	IGNA:	L PE	PTID:	E) ;	a .	**************************************
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	50			60			(70			80			90
a ጥC	- Carc		TCC	*	-	*		•	*		*		*	•
Met	Val	GTG	Trn	Ala	CTG	TGC	CGG	CTC	GCC	ACA	CCG	CTG	GCC	AAG Lys>
	a	a_EL	K-L	ECTO	DOMA	Cys IN 1	(WT	ים איז ים איז	TCNA	TUL	מדדים מדידים	Leu Leu	Ala	Lys>
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	a Deu	a EL	K-I.	ECTO:	Ser	TYP	Ser	Ser	Leu	Asn	Pro	Lys	Phe	Leu>
			-` ~	DC 1 0.	DOI:11	TIA T	(MI	in S.	LGNAI	L PE.	PTID	E)	a	a>
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AGT	GGG	AAG	GGC	TTG	GTG	ATC	TAT	CCG	AAA	TTA	GGA	GAC	AAG	CTG
Ser	а СтЛ	Dys a Fil	GIY	Leu	var.	Ile	Tyr	Pro	Lys	Ile	Gly	Asp	Lys	Leu>
	<u> </u>	a_LD	K-D .	EC 101	JORIA.	TM T	(WI.	rn S.	LGNAI	J PE	PTID	E)6	a6	a>
		19	90 -		2	200			210			22	20:	
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GAC	ATC	ATC	TGC	CCC	CGA	GCA	GÀA	GCA	GGG	CGG	CCC	TAT	GAG	TAC
Asp	Ile	Ile	Cys	Pro	Arg	Ala	Glu	Ala	Gly	Arg	Pro	Tyr	Glu	Tyr>
	a	a_EL	K-L 1	ECTO	OMA:	CN 1	(WIT	CH S:	[GNA]	J. PE	PTID	E)a	ae	a>
1	230			240			25	50 ga		•	260			270
	•		*	*.		*		•	*		* .		*	*
TAC	AAG	CTG	TAC	CTG	GTG	CGG	CCT	GAG	CAG	GCA	GCT	GCC	TGT	AGC.
Tyr	Lys	Leu	Tyr	Leu	Val	Arg	Pro	Glu	Gln	Ala	Ala	Ala	Cys	Ser>
	a	a_ELI	K-1 1	ECTOI	OMA]	EN · 1	(WIT	rh si	IGNAI	PE	PTIDI	E)a	ةـــنــة	a>
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Thr	Val	Leu	Asp	Pro	Asn	Val	Leu	Val.	Thr	Cys	Asn	Arg	Pro	Glu>
	a6	a_ELF	(-L E	ECTOR	IAMO	N 1	(WIT	H SI	GNAL	PE	PTIDE	E)a	ةــــــــــــــــــــــــــــــــــ	a>
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CAG	GAA	ATA	CGC	TTT	AÇC	ATC	AAG	TTC	CAG	GAG	TTC	AGC	CCC	AAC
Gln	Glu	Ile	Arg	Phe	Thr	Ile	Lys	Phe	Gln	Glu	Phe	Ser	Pro	Asn>
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TAC	ATG	GGC	CTG	GAG.	TTC	AAG	AAG	CAC		GAT	TAC	74T	ATT	- -
Tyr	Met	Gly	Leu	Glu	Phe	Lys	Lys	His.	His	Asp.	Tyr	Tyr	Ile	Thr>
	·	_ELK	-L E	CTOD	OMAI	N 1	(WIT	H SI	GNAL	PE	TIDE	E)a	a	·>
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Fig.14B.

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														GCC
														Ala>
	a	a_ELI	K-L	ECTO	DOMA.	IN 1	(WI	TH S	IGNA	L PE	PTID	£} <u>:_</u> ;	a	a>
	590			600			6	10			620	•		630
	•	•	•	*			٠.	*	: *	,	. *	•	*	*
CĊT	GGT	AGT	CGG	GGC	TCC	CTG	GGT	GAC	TCT	GAT	GGC	AAG	CAT	GAG
Pro	Gly	Ser	Arg	Gly	Ser	Leu	Gly	Asp	Ser	Asp	Gly	Lys	His	Glu>
<u> </u>	a	a_EL	K-L	ECTO	DOMA	IN 1	(WI	TH S	IGNA	L PÉ	PTID	E)	a	à>
		6.	10			550			660			٠.	70 ·	,
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ACT	GTG	AAC	CAG	GAA	GAG	AAG	AGT	GGC	CCA	GGT	GCA	AGT	GGG	GGC
Thr	Val	Asn	Gln	Glu	Glu	Lys	Ser	Glý	Pro	Gly	Ala	Ser	Gly	Gly>
	a	a_EL	K-L	ECTO	DOMA.	IN 1	(WI	TH S	IGNA	L PE	PTID	É)	a	a <u></u> >
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,	*		*	4		*	/(*	*		*		*	120
AGC	AGC	GGG	GAC	CCT	GAT	GGC	TTC	TTC	AAC	TCC	ÀAG	ĠGC	CCG	GGT
Ser	Ser	Gly	Asp.	Pro	Asp	Gly	Phe	Phe	Asn	Ser	Lÿs:	>	(
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·	TAC TAC	AAG	CTG	TAC	CTG	GTG	CGG	CCT	GAG	CAG	GCA	GCT	GCC	TGT
	Tyr Tyr	Lys	Leu	Tyr	Leu	Val	Arg	Pro	Glu	Gln	Àla	Ala	Ala	Cys>
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	Gly Gln	Asp.	Pro	Asn	Ala	Val	Thr	Pro	Glu	Gln	Leu	Thr	Thr	Ser>
	c	cc	EL	K-L	ECTC	DDOMA	IN 2	2 (NC	SIC	SNAL)		°	:c	>
	•	118	0	. •	11	90		. 1	200		. *	121	0	•
31	AGG CCC	AGC .	AAG (GAG	GCA	GAC	AĄC	ACT	GTC	AAG.	ATG	CCC	ACA	CAG
	Arg Pro	Ser	Lys	Glu	Ala	Asp	Asn	Thr	Val	Lys	Met	Ala	Thr	Gln>
	c	cc	EL	K-L	ECTC	DOMA	TM 2	: (NC	, 210	NALI		:c	: c	
	c	cc		K-L 230	ECTC	DOMA	124		, 510		c :50	:c		260
***	1220		. 1:	230		•	124	0		12	.50 •		. 1	260
***	c	GGT .	1 AGT (Ser	230 CGG Arg	GGC Gly	TCC	124 CTG Leu	O GGT	GAC Asp	12 TCT Ser	SO GAT ASP	GGC G1 y	AAG Lys	260 • CAT His>

Fig. 14	4 D		12	70		1	280		:	1290			130	00	
1 19.1				. •	. •		•		•	*		•		*	•.
	GAG	ACT	GTG	AAC	CAG	GAA	GAG	AAG	AGT	GGC	CCA	GGT	GCA	AGT	GGG
															Gly>
	 '		C	CE.	LK-L	ECT	ODOM.	AIN :	2 (N	o si	SNAL)(c		= <u> </u>
	13	310			1320			13:	30		1,3	340		. :	1350
	GGC	AGC	AGC	GGG	CAC	CCT	C D TT	GGC	ananca *	mmC *	N N C	TICC	***	CCC	
	Gly	Ser	Ser	Glv	Asp	Pro	Asp	Gly	Phe	Phe	Asn	Ser	LVS	• GGC	CCG
								2 (NO							
															Pro>
													•		d>
-			13	60		1.	370			1380			139		
		*	~~	* .		**	* .		*	., *.		•	133	. *	*
•	GGC	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC
	Gly>									•		•			
• • • • • • • • • • • • • • • • • • • •	·>			_			-						•		
	, .														Cys>
¥			·		e	en	UMAN	166.	ı FC	TAG	 '	e	е	e	e>
•	14	00.		:	1410	•		142	20	1	14	130		:	1440
		*		•	*		*		*	•	• ••	*		*	*
															CCC
	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro>
		·	e,	e.—	E	_HOM	AN I	SGL I	C 1	A.G	''	e	ee	e	e>
	•		145	50	· · ·	14	160	- 9		L470		٠.	148	30	
		•		*	• •. . : {\}-		•	. 1.	*	*		*		*	• .
															GTC
															Val> e>
			``````````````````````````````````````				, 11		15		'	'ـــــــــــــــــــــــــــــــــــــ	=	'	·
	14	90	. '. :	3	1500		• :	151	0		15	520		:	1530
		*		•	•		: *		•	*		•	•	*	*
															AAG
															Lys> e>
•			. 3. ;			# 1		) ¥		* •			·\		·
			154	10		15	550			L560			157		•
		6.J.T.	mác	* :	CTC	CNC	*			*				*.	*
•															ACA Thr>
4.															e>
	,								31 .	·					
•	15	80			590	5.5		160	0 /	- 4 f f	16	510		:	1620
	N. C.	ccc		iki Caci			_				m> 0	*		*	*
	LVS	Pro	Ara	Clu	Glü	Gla	TÜT	ASD	Ser	Thr	TAC	ÁŤ	Ual	Ual	AGC Ser>
	e	€	e	6	<u> </u>	HUMA	M IC	G1 F	CT	۸G6	26	2. (	5 .6	>	e>
		· /.													
	$, d_{2}$		163	0	18.5, 1	16	40	,	1 1	1650	:.		166	50	_
•			ACC								ጥፈል		220	GAC	TAC
April 1	Val	Leu	Thr	Val	Leu	His	Gln	ASD	Trp	Leu	Asn.	G1 v	Lvs	Glu	Tyr>
	е	6	6	e	ুর্ন্তি ব	ними	N IC	GiF	CT	₹	≥(	e(	=,,_ ee	=	e>

# Fig.14E.

		3.	•		•									
167	0			1680			169	90		1:	700			1710
	*		*	*		*		*	*		*		*	*
AAG T	GC .	AAG	GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA
ras C	ys :	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	LVES
e_	е		e	e	_HUM	AN I	GG1	FC T	AG	e	≥	ë	a	e>
		172	20		1	730		•	1740			175	30	
	*		*	*		*	•	*	*		*		*	*
ACC A	TC '	TCC	AAA	GCC	AAA	GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC
Thr I	те :	ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr>
e_	е.	<u></u>		e	_HUM	AN I	GG1	FC T	AG	ee	<u> </u>	ee	<u> </u>	e>
176	n			1770			170							
	*		*	1770	+ 3		178	30		17	790			1800
ACC C	TG (	200	CCA	TYCE	CGG	CDT	CNC	Caxc -	NCC.	N N.C.			~~~	
Thr L	eu j	Pro	Pro	Ser	Ara	Asn	Glu	Leu	Thr.	Tare	AAC	CAG	GTC	AGC
e_	e_		<u></u>	е	HUM	N I	3G1 1	FC T	AG.	e e	Man	GTII	vaı	ser>
			. 7							<u> </u>	·`	٠ر	·'	=>
		181	.0		18	320			1830			184	10	
	<b>k</b>		<b>*</b>	*	-2	*		÷	*		*		*	*
CTG A	CC 7	rgc	CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC.	GCC	GTG
Leu T	hr (	Cys	Leu	Val	Lys.	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala-	Val>
e_	e_	<u></u> -€	}	<u> </u>	_HUM/	'n IÇ	3G1 I	C T	AG	ee	:	<u>≥∷_</u> €	<u> </u>	e>
							٠.					,		,
1850	)		. 1	L860	!		187	70		18	80			1890
CNC M	20 c		* _	*		*		*	*		*		*	*
GAG TY	- C	sAG	AGC	AAT	GGG.	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC:	ACG
Glu T	י עי	a i ir	ser	Asn	GIA	GIN	`hro	GIU	Asn	Asn	Tyr	Lys	Thr	Thr>
		·e		=	_nom	TA TO	GI I	C 12	AG(	<u>-</u>		9€	<u></u>	e>
		190	O ,		19	10	20.20	1	1920			100	^	
•	r		<b>*</b> ,	*		*	4	* ;	LJ20		*	193	•	
CCT CC	C G	TG	CTG	GAC	TCC:	GAC	GGC	TCC	TTC	שירי.	כיזיני	ጥልሮ	y CC.	AAC
Pro Pr	o V	al	Leu	Asp	Ser.	Asp	Gly	Ser	Phe	Phe	Leu	Tvr	Ser	Tues
е	_e_	е	ع_تنت_6		HUMA	N IC	G1 F	C T	\G6	ee				. >
			•			•								
1940	)	7	1	950	Au.		196	0 :	£ .	19	70 ·	1.	1	.980
*			<b>*</b>	*	ŧ.	*	٠.	<b>★</b> , st	*	S	*		*	*
CTC AC	CG	TG	GAC	AAG	AGC .	AGG:	TGG	CAG	CAG	GGG.	AAC	GTC	TTC -	TCA
Leu Th	IF, V	aı,	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe.	Ser>
e_	e_	е	е		HUMA	N. IG	G1 F	C TA	\G€	ее	e	e	е	>
		1996	n .	•	20	00	15	2	010	Ø1 s		منم		
		100	• .			*		* =	010	(FF )		202	0	
TGC TC	C G													
Cys Se	r V	al I	Met:	His.	Glu /	Ala	Leu	His.	Asn	Hie	Tare	The -	CAG	AAG
e	_e_	e	e	1.5	HUMAI	N IG	G1 F	C TA	G e		- y +	- III	:;;;a	ny 5>
2030	i, 1		2	040	·		205	0 👉	C			4 °.		
*		•												
AGC CT														
Ser, Le												4434	4 17.A.	f. 15
е	н	UMAI	i IG	31 F	CITAC	3 <u> </u>	е	<u>·</u> >,			• • •	:		

# Fig.15A.

	_					
	10		20	30		40
*	*	•	•	* *	•	
ATG GCC ATG	GCC C	GG TCC	AGG AGG	GAC TCT	GTG TGG	AAG TAC TGT
Met Ala Met	Alab	ra Ser	yea yea	Aco Ser	Val Tro	Lys Tyr Cys>
י בטומו		remo per	MIY MIY	Map ser	var irb	ras lar cass
aEPHKI	.N-DZ	ECTO DO	MAIN I	(MITH SI	GNAL PEP	TIDE)a>
50		60		70	80	90
	*	*	•	* *	*	, <b>4</b>
TGG GGA CTT	TTG A	TG GTT	TTG TGC	AGA ACT	GCG ATC	TCC AGA TCG
						Ser Arg Ser>
						rIDE)a>
			-	,		
٠ 1	.00	1	.10	120		130
•	.00			120		130
AMA COM OMA						
						TCC AAA TTT
						Ser Lys Phe>
a_EPHRI	N-B2	ECTO DO	MAIN 1	(WITH SI	GNAL PEP	ride)a>
140	1	.50	1	60	170	180
, <b>*</b> .	<b>★</b> - 27	•	*	- 4 · · · · · · · · · · · · · · · · · ·	•	•
CTA CCC GGA	CAA G	GC CTG	GTA CTA	TAC CCA	CAG ATA	GGA GAC AAA
						Gly Asp Lys>
aEPHKI	IN-DZ	ECTO DO	MAIN I	(MITH SI	GNAL PEP	ride)a>
]	90	2	200	. 210	•	220
*	*	*	. •	* *	*	•
						GTT GGC CAG
Leu Asp Ile	: Ile C	ys Pro	Lys Val	Asp Ser	Lys Thr	Val Gly Gln>
						TIDE)a>
230	2	40	. 2	50	260	270
•		*	. –	* * *	*	
תאת כאא תאת	י אידי אידי א	את כייייי	מאת אתכ	COT CAM	200 000	CAA GCA GAC
						Gln Ala Asp>
a_EPHRI	N-B2	ECTO DO	MAIN 1	(WITH SI	GNAL PEP	rIDE)a>
2	280	. 2	90	- 300		310
	<b>*</b> 9	*	•	• •	•	•
AGA TGC ACA	ATT A	AG AAG	GAG AAT	ACC CCG	CTG CTC	AAC TGT GCC
Arg Cys Thr	: Ile L	ys Lys	Glu Asn	Thr Pro	Leu Leu	Asn Cys Ala>
						TIDE)a>
320	3	30 .:	٠ . ء	40	350	7-4- 360
*	*	*	•		330	* *
ACA CCA CAC		'AM CMC	A A A MMC	ACC AMC	tak a ci a manini	
						CAA GAA TTC
						Gln Glu Phe>
a_EPHRI	LN-B2	ECTO DO	MAIN 1	(WITH SI	GNAL PEP	ride)>
			•			
3	70	3	80	390	\$. \$ ** \$	400
•	•	*		• •	*	* *
AGC CCT AAC	CTC T	GG GGT	CTA. GAA	TTT CAG	AAG AAC	AAA GAT TAC
						Lys Asp Tyr>
						ride)a>

Fig. 15B.

410	3.	420	•		4	30		. 4	140			450
ተልር ልጥጥ	** ATA TC1	•	mc s	•	000	*	*	212	*		•	•
TAC ATT												
Tyr Ile a_EP	ile Ser HRIN-B?	Thr	Ser .	Asn Matn	Gly	Ser	Leu	Glu	Gly	Leu	Asp	Asn>
			10 00	.TUTI	• 1	(44 T T I	1 310	SIAVE	FEP.	LIDE		·>
•	460		4	70			480			49	90	
CAG GAG	.GGA GGC	GTG	ተርር (	CAG	ACA	AGA	GCC	ΑΫ́С	AAG	ATC	CTC	λπc
Gln Glu	Gly Gly	/ Val	Cys	Gln	Thr	Arg	Ala	Met	Lys	Ile	Leu	Met>
aEF	PHRIN-B	EC.	TO DO	MAİN	1 1	(WITI	d SI	GNAL	PEP'	ride	)	a>
500		510			5	20		9	530			540
◆.		•		•		*	•		•		. •	•
AAA GTT	GGA CA	GAT	GCA .	AGT	TCT	GCT	GGA	TCA	GCC	AGG	AAT	CAC
Lys Val	HRIN-B	L ASP	ALA TO DO	Ser Math	ser J 1	(WIT)	GLY	Ser SNAI.	Ala	Arg	Asn :	His>
					` -		•					
•	550		5	60		4	570		_	58	30	
GGT CCA	ACA AGA	CGT	CCA		СТА	GAA	-	CCT	ACA	ААТ	GGG	ACA.
Gly Pro	Thr Arg	, Arg	Pro	Glu	Leu	Glu	Ala	Gly	Thr	Asn	Gly	Arg>
aEF	HRIN-B2	EC.	TO DO	MAIN	1 1	(WITI	I SI	<b>SNAL</b>	PEP	ride:	)	a>
590		600	• •		6	10	٠.,	٠.	520			630
•	•	•		*	,	* :	•		•	,	•	•
AGT TCA	ACA ACA	AGT	CCC .	TTT	GTG	AAG	CCA	AAT	CCA	GGT	TCT	AGC
Ser Ser a_EF	HRIN-B	Ser EC	TO: DO	PNE MATN	11 Val	Lys (WIT)	Pro:	ASD JANE	PED	Gly	Ser	Ser>
						,						
	640		6:									
ACC GAT	1.4											999
Thr Asp	Gly Asr	Ser	Ala	Gly	His	Ser	Gly	Asn	Asn	Leu	Leu	Gly>
aEP	HRIN-B2	EC.	TO DO	MAIN	1 1:	(WITH	i si	GNAL	PEP	ride:	)	a>
680		- 690	, 4	· 14	7	0.0	·		710			720
•	•		و در د	*		13 Ja	y .*.	. 1 . 44	i 🔩 😘		•	•
GGC CCG	GGA ATA	GTT	,TŢA, (	GAG.	CCT	ATC	TAC	TGG	TAA	TCC	TCG	AAC
bb	<del>-</del>	•				2. ₂ .	· ·					
¥ d	Ile	val	Leu	Glu	Pro	.Ile	Tyr	Trp	Asn	Ser	Ser	Asn>
i	· · <u></u>	_ЕРНК	TN-B5		TO .	DOMA:	IN 2	. ( W	ITHO	UT S		>
	730	. •			-		750		,	76	_	
TCC AAA	* יייי רייא	•		•		*	•		*	001	*	*
Ser Lys	Phe Leu	Pro	Gly (	Gln	Gly	Leu	Val	Leu	Tyr	Pro	Gln	Ile>
EPHR	IN-B2	ECTO	DOMA:	IN 2	(, )	WITHO	ידט(	IGN	AL PI	EPTII	ÞΕ)_€	<u></u> >
770 ^{- 3}	Token Madij	780	· * 47.	. :*	76	0	i. Gundani	7 7 CT	100	. 313	•	810
												•
GGA GAC	AAA TTG	CAT	ATT A	ATT.	TGC	CCC,	AAA	GTG	GAC-	TCT	AAA	ACT
Gly Asp EPHR	Lys Leu IN-B2	ECTO	DOMY:	LTE. LTE.	Cys	Pro VITHO	Lys our o	Val SIGNI	Asp. •q ≀t.	Ser	Lys	Thr>
	IN-B2											

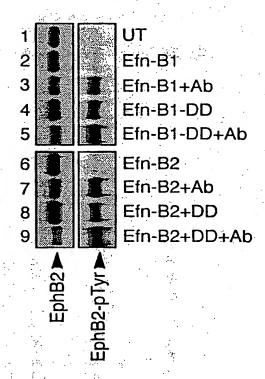
Fig.150	<b>D.</b> .	820	•	830	•	840	•	850	•
	GTT GG				AAA GTT				
	EP	RIN-B2	ECTO	DOMAIN	2 ( WITH	OUT SIGN	IAL PEPT	IDE)_e	·> ·
	860		870		880		890		900
					AAG AAG				
-					Lys Lys 2 ( WITH				
		910		920 *		930 .	· <b>+</b>	940	
•					GAT GTG				
				_	Asp Val 2 ( WITH	-			
	950		960	•	970	•	980	•	990
					TGG GGT				
					2 ( WITH			_	•
•	•	1000	)	1010	•	1020	1	030	
					ACA TCA				-
. ,		TYT 1 HRIN-B			Thr Ser 2 ( WITH				
	1040		1050		1060		1070	1	1080
	CTG GA	AAC O	* CAG GÁG	GGA GGC	• GTG TGC	* CAG AC	* A AGA GO	* C ATG	AAG
	Leu As		Sln Glu	Gly Gly	Val Cys 2 ( WITH	Gln Th	r Arg Al	a Met	Lys>
•	•	1090	•	1100	•	1110	. 1	.120	•
					A GAT GCA A Asp Ala				
					2 ( WITH				
	1130	tros er e le	1140		1150°		1160		1170
	AGG AA	CAC C	GT CCA	ACA AGA	CGC CCA	GAG CT			
					Arg Pro 2 ( WITH				
· .	e, **•)	1180	) (* ) • 1840 •	1190		1200		210	•
					AGT CCC				
÷ .					Ser Pro 2 ( WITH	OUT SIG			
	এ ৯৬% ১০	21 397			1240			•	260
					AGC GCG Ser Ala				
					2 ( WITH				

Fig.15	5D.	12	70		:	1280			129	0		13	00	
	CTC CTG	GGG	G GG	ccc	GGG									CAC His>
		•	-	y Pro		y>	_c							c>
	Leu Leu	_	Xxx:	_d	_d	_>							•	
	1310			1320			133	30		··· 13	40		1	350
• .:	ACA TGC													
*	c	c <u></u> (	°(		_HUM/	AN I		FC T	AG			=c	:c	
	•	136	50		1.	370			1380		_	139	0	_
÷	GTC TTC	Carc	ጥጥር	CCC	CCA	מ מ.מ	CCC	AAC	GÁC	ACC	C.T.C.	ATIC	» ~~	TYCC
	Val Phe													
												=		
. ;					٠.					٠.:	٠			
	1400		_ ;	L410		_	14:	20.		14	130		. 1	440
	CGG ACC	CCT	GAG	CTC	אכא	4CC	CTC	CTC	CTC.	CAC	CIVC	NCC	CAC	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
	Arg Thr													
														>
•)(•	. •			٠				: .	, (::		. :			
		145	50	-41	14	160			1470		•	148	Ö	
•	CAC CCM	CNC	. *	*		*		*	*	22.0	-		*	*
1,	GAC CCT Asp Pro													
:														
to a constant of the constant				4.4					1, 7	J. 13 5 1	·`			
	1490		. 1	1500	٠.			r.o		15	20		1	530
			*	* *		*	9 V.		*		. •		* .	*
	CAT AAT His Asn c	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Ġlu	Gl'n	Tyr	Aśn	Ser	Thr>
	,											3# Z		
: .		154	10	•								157	0	_
×	TAC CGT	CAC	CAC			CMC	NCC						. <del>.</del>	CMC
•	Tyr Arg													
	c													
. '1	1580		. 1	590	ny s	•	160		Jaros Parist	16	10	. • <del>•</del> • • • •	. 1	620
	AAT GGC													
	Asn Gly													
•		·—-			_HUMA	AIN IC	JGI I	·C 17	AGC	c	·	c	.—_c	>
	•	163	30	4	16	40		•	1650		•	166	0	•
	ccc ccc													
	Ala Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg>
	c	<b>c</b> (		2	HUMA	AN IC	GG1 E	C T	AG d	: 0	: (			· >

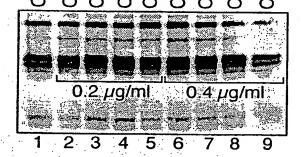
# Fig.15E.

	<u> </u>												
1670			1680			169	90		17	700			1710
		•	•		*		*	•		•		•	*
GAA CC	A CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG	ACC
Glu Pr	o Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Ara	Asp	Glu	Leu	Thr>
c_	_c	.c	c	_HUM	AN I	GG1 1	FC T	AG	c (	c (	c	3 (	c >
				_									
	17	20		17	730			1740			175	0 6	
•		• '	*		•		•	*		*		*	*
AAG AA	C CAG	GTC	AGC	CTG	ACC.	TGC	CTG	GTC	AAA	GGC	TTC	TAT	CCC
Lys As	n Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro>
c	_c	c	c	_HUM	AN I	GG1	FC T	AG	c(	c	هه	·	c>
	•										* .		
1760	• : .	:	1770			170	80		1	790		(	1800
•		. •			*		Ŕ,	*		<b>★</b> 3		*	•
AGC GA	OTA O	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC
Ser As	p Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn>
c	_c	.c	c	HUM	AN I	GG1	FC T	AG	c	c(	c	3	c>
·									. :				
	18	·		18	320			1830			184	10	
•			*		•		•	*	•	*		*	*
AAC TA	C AAG	ACC	ACG	CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC
Asn Ty	r Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe>
c_	_c	C	c	_HUM	AN I	GG1	FC T	AG	c	c	٠٥	3	c>
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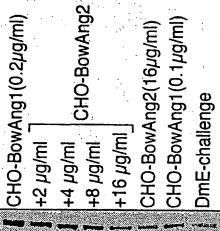
Fig.16.

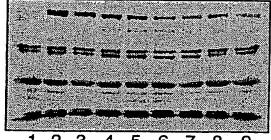


CHO-Ang1*(0.4µg/ml)
CHO-BowAng1#990805A
CHO-BowAng1#990805B
CHO-BowAng1#990630
CHO-BowAng1#990805A
CHO-BowAng1#990805A
CHO-BowAng1#990805A
CHO-BowAng1#990805B









## INTERNATIONAL SEARCH REPORT

in. .ational Application No PCT/US 99/30900

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A. CLASSI IPC 7	FICATION OF SUBJECT MATTER C12N15/12 C12N15/62 C12N5/1 C07K14/52	.0 C12N1/21 C07	(14/515
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Minimum do IPC 7	ocumentation searched (classification system followed by classification country CO7K	tion symbols)	
	tion searched other than minimum documentation to the extent that		
Electronic d	ata base consulted during the international search (name of data b	ase and, where practical, search terms use	d)
		•	
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the re	elevant passages	Relevant to claim No.
			<u> </u>
<b>X</b>	WO 96 37621 A (MORPHOSYS PROTEINOPTIMIERUNG ; PACK PETER ( ADOLF (DE)) 28 November 1996 (19		1-5, 12-22
	abstract page 1, line 12 - line 15 page 2, line 4 - line 9 page 14, line 6 - line 11 page 16, line 29 - line 34 figure 1A		0.11
A			8-11 6,7
		_/	
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1	·		
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X Furti	l her documents are listed in the continuation of box C.	Patent family members are liste	d in annex.
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	ent defining the general state of the art which is not	or priority date and not in conflict wit cited to understand the principle or t	
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· which	is cited to establish the publication date of another n or other special reason (as specified)	"Y" document of particular relevance; the cannot be considered to involve an i	claimed invention
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"P" docume	ent published prior to the international filing date but	in the art.  *8.* document member of the same pater	
<b> </b>	nan the priority date claimed actual completion of the international search	Date of mailing of the international s	
2	2 May 2000	09/06/2000	
Name and r	nailing address of the ISA	Authorized officer	
	European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,	Galli, I	
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Fax: (+31-70) 340-3016

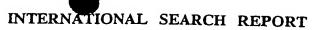


#### INTERNATIONAL SEARCH REPORT

In. atlonal Application No PCT/US 99/30900

0.40	Aller OCCUMENTS CONCINCING TO BE DESCRIPTION	T PC1/05 99	
	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	<del></del>	· .
Category *	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.
X	WO 92 03569 A (SANGSTAT MEDICAL CORP) 5 March 1992 (1992-03-05) abstract page 1 -page 3 page 19, line 14 - line 23		23-26, 29,32-41
Y A	page 13, Time 14 Time 23		30 27,28
Y	WO 95 27060 A (REGENERON PHARMA) 12 October 1995 (1995-10-12) cited in the application abstract page 23, line 21 -page 24, line 8 claims 4,8		8-11,30
<b>\</b>	EP 0 816 510 A (TORAY RESEARCH CENTER INC ;TORAY INDUSTRIES (JP)) 7 January 1998 (1998-01-07) abstract		1–22
Α	DAVIS S. ET AL.: "Isolation of Angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning" CELL, vol. 87, 27 December 1996 (1996-12-27), pages 1161-1169, XP002138354 cited in the application the whole document		6,7,27, 28
4	MAISONPIERRE P.C. ET AL.: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 55-60, XP002138355 cited in the application the whole document		6,7,27, 28
1	TOURNAY C. ET AL.: "Uptake of recombinant myeloperoxidase, free or fused to Fc-gamma, by macrophages enhances killing activity towards micro-organisms" DNA CELL BIOLOGY, vol. 15, no. 8, 1996, pages 617-624, XP000907279 abstract page 618		21,40
			:.

Form PCT/ISA/210 (continuation of second sheet) (July 1992)



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Information on patent family members

In atlonal Application No PCT/US 99/30900

Patent document cited in search report		Publication date	1	Patent family member(s)	Publication date
WO 9637621	Α	28-11-1996	CA	2222055 A	28-11-1996
			EP	0827544 A	11-03-1998
			JP	11508126 T	21-07-1999
WO .9203569	Α	05-03-1992	CA	· 2090105 A	01-03-1992
			EP	0547163 A	23-06-1993
			JP	6502301 T	17-03-1994
			US	5672486 A	30-09-1997
WO 9527060	Α	12-10-1995	US	5747033 A	05-05-1998
		•	AU	691915 B	28-05-1998
			AU	2278995 A	23-10-1995
			CA	2187167 A	12-10-1995
			EP	0758381 A	19-02-1997
			JP	9511401 T	18-11-1997
			ZA	9502762 A	20-02-1996
EP 0816510	A	07-01-1998	ĊA	2213512 A	03-07-1997
			WO	9723639 A	03-07-1997

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